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13. ABSTRACT *(Maximum 200 Words)*

The primary accomplishments of the previous funding period were: 1) cloning and expression of the mouse isoform of Scn10a gene product (SNS; TTX-resistant sodium channel); 2) functional heterologous expression and initial biophysical characterization of the mouse Scn10a gene product in sympathetic neurons; 3) cloning and sequencing of 4 Kbp of upstream genomic DNA; and 4) identification of BAC (bacterial artificial chromosome) clones harboring the mouse Scn10a gene. The nucleotide sequence of the mouse Scn10a cDNA obtained in these studies deviates significantly (170 discrepancies in 5874 bases) from a previously published cDNA sequence derived from genomic DNA (GenBank accession number Y09108) and thus represents new information. Heterologous expression of the Scn10a cDNA in sympathetic neurons revealed a significant depolarizing shift in the biophysical properties of the expressed channel when compared with natively expressed channels. These findings indicate that an accessory protein may influence the behavior of the channel. A candidate promoter region for the Scn10a channel has been cloned and identified in BAC clones. Taken together, these results provide the experimental foundation for our future studies aimed at identifying new potential therapeutic targets that modify the function and expression of sodium channels involved in pain transmission.

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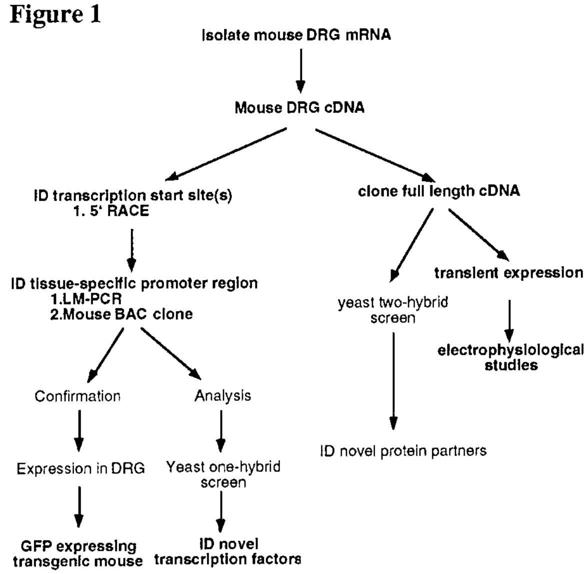
Introduction

The Scn10a gene product encodes a tetrodotoxin-resistant sodium channel (SNS/PN3) expressed exclusively in a subset of primary sensory neurons (e.g., dorsal root and nodose ganglia) believed to be involved in pain transmission (Akopian et al., 1996). Thus, it is important to understand mechanisms contributing to both the function of the protein and the exquisite specificity of gene expression. The overall research plan is detailed in the flowchart depicted to the right. During the last funding period, we have made significant progress on both the genomic (left branch) and proteomic (right branch) sections of the research plan.

Figure 1



Specifically, we have cloned and sequenced the full length mouse Scn10a cDNA. Moreover, we have demonstrated that the cDNA is functionally expressed in neurons and have initiated biophysical characterization of the expressed channels. With regard to progress toward better understanding the regulation of Scn10a transcription, the putative transcription start site has been identified using 5' rapid amplification of cDNA ends (RACE) and 4 Kbp of upstream genomic region has been sequenced using ligation-mediated PCR (LM-PCR). Finally, bacterial artificial chromosome (BAC) clones containing the full length mouse Scn10a gene have been identified.

Body

A. Cloning of mouse Scn10a cDNA

In order to obtain a murine Scn10a clone, dorsal root ganglion (DRG) neurons were enzymatically dissociated from adult CD1 mice and polyA mRNA isolated by standard techniques. A full length mScn10a clone was amplified from this material by RT-PCR using oligonucleotide primers based on the genomic sequence data provided in Souslova et al. (1997). The open reading frame was subcloned into the mammalian expression vector pCI (Promega) and sequenced with an ABI 377 automated sequencer (Appendix A). The sequence of the Scn10a clone was verified by sequencing the PCR product from several different mRNA preparations.

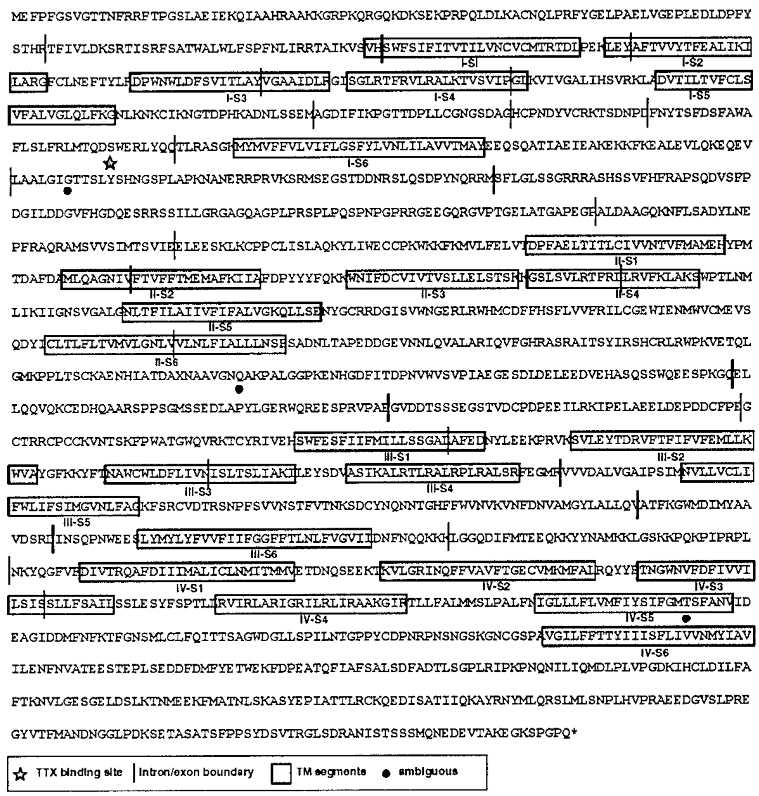
The nucleotide sequence of mouse Scn10a cDNA, when compared with sequence (#Y09108) derived from mouse genomic data (Souslova et al., 1997), revealed 170 discrepancies of 5874 total bp (Appendix B). About 50% of the changes do not alter the primary sequence (i.e., are silent mutations). About 1/3 of the "polymorphisms" are A to G changes (genomic to cDNA). An alignment of the primary amino acid sequence of our

clone compared with #Y09108 is shown in Appendix C. The reason for these discrepancies is unclear.

The derived primary sequence of mouse Scn10a is 93, 82, and 81% identical to the rat (X92184), human (AF117907), and dog (U60590) Scn10A sodium channel, respectively. When conserved substitutions are taken into account (ClustalW analysis), the sequence similarity is 95, 89, and 88%, respectively.

Figure 2, Mouse Scn10a primary amino acid sequence

Putative transmembrane segments are shown in shaded boxes with each color representing one of four homologous domains. The serine residue shown to be involved in TTX insensitivity in the rat ortholog (Sivilotti et al., 1997) is marked with a star. Putative intron/exon boundaries are from Souslova et al., 1997. Ambiguous residues are those not confirmed by sequencing of different clones.



B. Expression of Scn10a cDNA clone in rat sympathetic neurons

Rat sympathetic neurons isolated from superior cervical ganglion (SCG) were used as a host for Scn10a expression. Previous studies have shown that natively expressed sodium channels in SCG neurons are completely suppressed by tetrodotoxin (Schofield & Ikeda, 1988). Scn10a (0.1–0.2 μg/μl) and EGFP (Clontech, 0.05 μg/μl) cDNAs were co-injected into the nucleus of SCG neurons using an Eppendorf microinjection system (Ikeda, 1996, 1997). Patch clamp recordings were made following 12-18 hours of incubation at 37 C. Successfully injected cells were identified by EGFP fluorescence using an inverted microscope (Nikon) equipped with an epifluorescence unit.

Voltage-clamp recordings were made at room temperature (23–25 °C) using the whole-cell variant of the patch-clamp technique. Solutions designed to isolate tetrodotoxin resistant (TTX-R) currents were as follows: external (mM): TEA-Cl 120, NaCl 50, HEPES 10, $MnCl_2$ 2, and glucose 10. TTX (1 μM) was added to suppress sensitive

currents. The pH of the solution was adjusted to 7.4 with TEA-OH. The osmolality of the solution was 327 mosm/kg. Internal (mM): CsCl 115, NaCl 10, EGTA 11, $CaCl_2$ 1,HEPES 10, MgATP 4, GTP 0.1, and diTRIS phosphocreatine, 5. The pH of the solution was adjusted to 7.2 with TEA-OH. The osmolality of the solution was 303 mosm/kg. Current-voltage (I-V) and activation curves were derived from currents evoked by a 20 msec test pulse to various potentials from a holding potential of -80 mV. Conductance calculations were made using the chord conductance equation assuming a reversal potential of +40 mV. Inactivation was determined using a 1 sec conditioning pulse followed by a test pulse to 0 mV. Parameters for activation and inactivation curves were determined by fitting the normalized conductance curve to a Boltzmann function using a non-linear regression program. An example of currents and activation and inactivation analysis is shown in Figure 3.

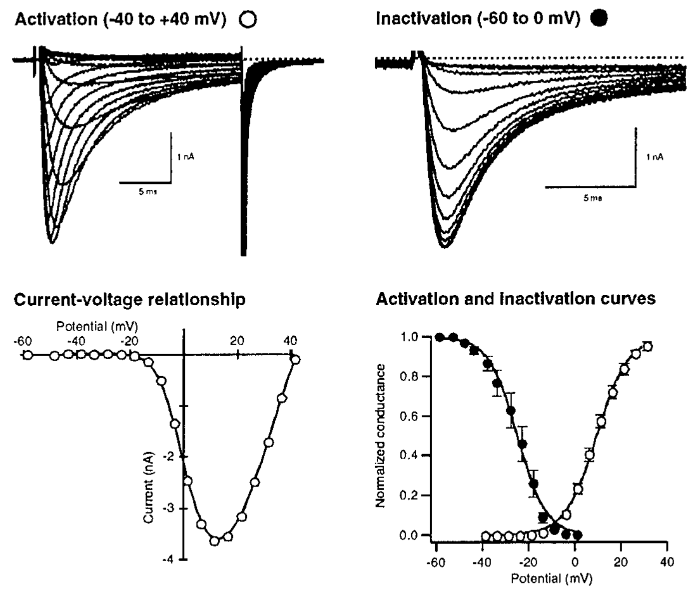


Figure 3. TTX-resistant sodium currents recorded from rat sympathetic neurons expressing Scn10a channels.

Upper right: Family of currents evoked by depolarizing pulses. *Lower left*: Current voltage relationship. *Upper right*: Currents evoked with the inactivation voltage protocol. *Lower right*: Steady state activation and inactivation curves.

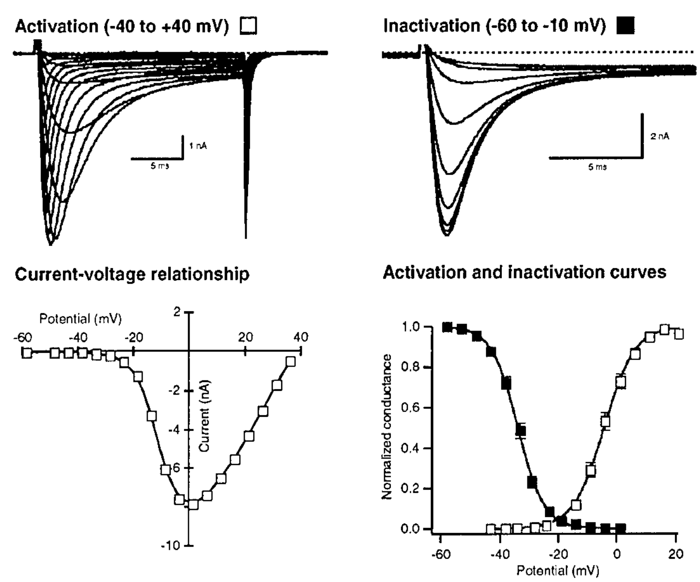


Figure 4. TTX-resistant sodium currents recorded from mouse DRG neurons

Upper right: Family of currents evoked by depolarizing pulses. *Lower left*: Current voltage relationship. *Upper right*: Currents evoked with the inactivation voltage protocol. *Lower right*: Steady state activation and inactivation curves.

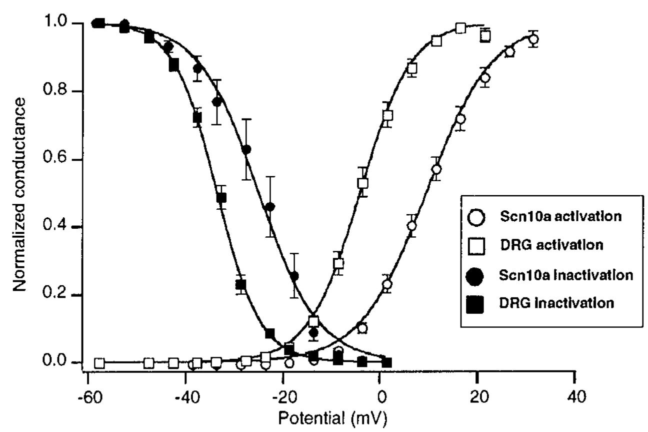
Tetrodotoxin-resistant sodium currents were also recorded from acutely isolated mouse DRG neurons. The goal of these studies was to compare the heterologously expressed channels with natively expressed channels. An example of such currents is shown above (Figure 4.)

Comparison of heterologously and natively expressed TTX-resistant sodium channels revealed that depolarizing shifts in both the activation and inactivation curves of the cloned channels. Analyses of individual neurons are included in the Appendix (D and E). Mean activation and inactivation curves are depicted in Figure 5.

Figure 5. Mean activation and inactivation curves for natively and heterologously expressed TTX-R Na^+ channels.

Activation and inactivation curves were fit to the following equation:

$$G(V) = \frac{Gmax}{1 + \exp[(V-Vh)/k]}$$



A summary of the mean activation and inactivation parameters derived from the nonlinear regression analyses is show in Table 1.

Table 1.

	Activation		Inactivation	
	Vh_{act} (mV)	k_{act} (mV)	Vh_{inact} (mV)	k_{inact} (mV)
DRG	-4.3 ± 0.9 (9)	5.0 ± 0.2 (9)	-34.0 ± 0.6 (9)	4.3 ± 0.2 (9)
Scn10a	9.4 ± 1.1 (9)**	6.4 ± 0.4 (9)**	-25.2 ± 2.2 (4)	5.6 ± 0.5 (4)

C. Identification of BAC (bacterial artificial chromosome) clones harboring the Scn10a gene

The absolute size of the promoter for the Scn10a gene is unknown. Since other Na^+ channel genes are regulated by promoters that span greater than 50 kb of genomic DNA, we reasoned that the Scn10a gene promoter may also be very large. To increase the likelihood of isolating the entire promoter, we chose to screen a mouse genomic BAC library prepared by Incyte Genomics. BAC (Bacterial Artificial Chromosome) libraries

are constructed in specialized plasmid vectors that stably maintain greater than 125 kb of contiguous cloned genomic DNA, and are well-suited for cloning projects where large stretches of genomic DNA need to be examined. Three BAC clones (#26462, #26463, and #26464) were identified in a PCR-based screen using oligonucleotide primers specific to exon 1 of the Scn10a gene.

The clones are currently being analyzed to determine the extent of 5'-flanking DNA (which contains the promoter) carried on each. Preliminary analysis of the three BAC clones by restriction endonuclease analysis indicates that the clones contain contiguous overlapping stretches of mouse genomic DNA containing an undetermined amount of exonic/intronic DNA and 5'-flanking DNA of the Scn10a gene. Pulsed field gel electrophoresis (PFGE) of the clones shows that they are similar, but not identical, in size and are greater than 150 kb (Figure 6). To determine the extent of 5'-untranslated DNA carried on each BAC clone, we are in the process of modifying each clone to contain a unique restriction site, SwaI and/or CeuI, just upstream of the ATG translation codon of the Scn10a gene. Digestion of the modified BAC clones at the unique NotI site in the pBeloBAC II cloning vector and the SwaI and/or CeuI site will release a segment of the BAC DNA corresponding to the 5'-flanking DNA, which will then be sized by PFGE. Incorporation of the SwaI and CeuI sites into each BAC clone is being done using a modified protocol first described in Yang et al. (1997).

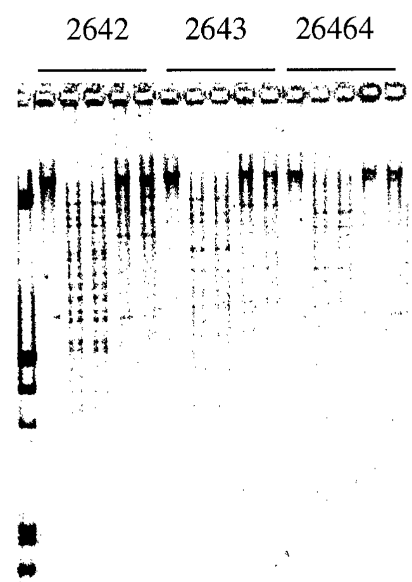


Figure 6. Restriction enzyme digestion of BAC DNA. Digestion of the three BAC clones with BamHI or SalI is shown. Lane 1 contains high-molecular weight λ markers

The modification of the BAC clones will also allow us to examine the Scn10a gene promoter in transfected primary neurons as the coding sequence for the enhanced green fluorescent protein (EGFP) will simultaneously be placed immediately downstream of the 5'-flanking region. Expression of the EGFP gene under control of the Scn10a gene promoter is expected to provide a very sensitive read-out of this promoter's activity. In this protocol, a specialized shuttle vector is first constructed *in vitro* that carries a small segment of the 5'-flanking sequence of the Scn10a gene fused to the EGFP gene in the shuttle vector PLD53PA (Figure 7). The shuttle vector is next recombined *in vivo*

adjacent to the 5'-flanking sequence of the Scn10a gene on the BAC clone, creating a Scn10a promoter-IRES I EGFP fusion in the BAC clone. We have determined the DNA sequence of approximately 900 bp of the 5-flanking region of the Scn10a gene by direct sequence analysis of one of the BACs (Appendix F). We have constructed appropriate pairs of forward and reverse oligonucleotide primers that will be used to amplify by the PCR an approximately 600 bp subfragment (Figure 8). This 600 bp subfragment will be subcloned into the multiple cloning site of the shuttle vector PLD53PA via the NotI and SwaI sites incorporated into the forward and reverse primers. Recombination of the shuttle vector into the BAC DNA will occur at the second IRES I EGFP-PA sequence in PLD53PA. The SacB gene in this vector provides for sucrose-counterselection to select for BAC recombinants that have undergone a recombination event resulting in a final BAC clone containing a stably-integrated Scn10a promoter-EGFP fusion. The resulting BAC clones will retain whatever 5'-flanking DNA that was carried on the unmodified BACs, only fused to IRES I EGFP.

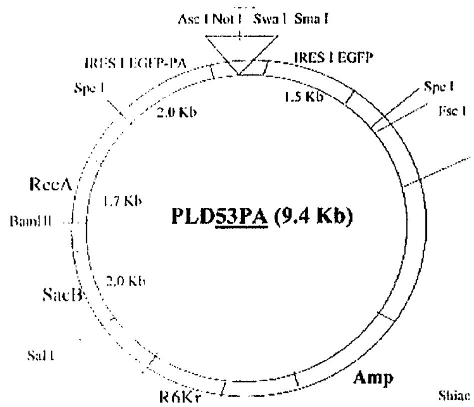


Figure 7. Map of the shuttle vector PLD53PA. The NotI/SwaI sites within PLD53PA for insertion of Scn10a DNA is shown.

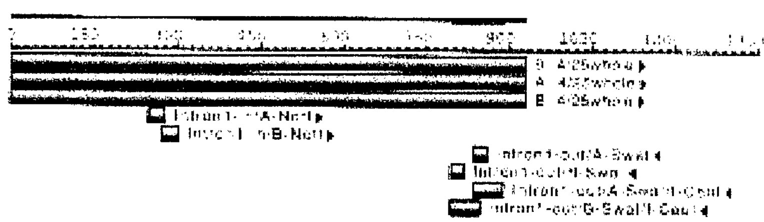
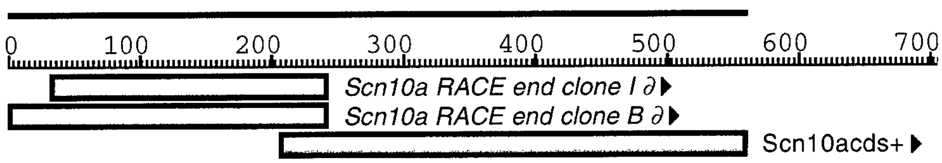


Figure 8. Schematic of the 900 bp 5'-flanking DNA and location of PCR primers. Sets of forward and reverse primers to amplify a 600 bp subregion for insertion into PLD53PA are shown. The specific restriction sites incorporated into each primer is indicated.

5' RACE Analysis was performed on mRNA isolated using the cellulose based SV total RNA and polyAtract® mRNA isolation systems from Promega. The mRNA was reverse transcribed to first strand cDNA using the Advantage™ RT for PCR kit from Clontech. The cDNA was then and used in the SMART™ RACE cDNA Amplification kit (Clontech) in which the general first strand cDNA synthesis step was followed by a single round of PCR with a universal adaptor primer and a gene specific primer. The gene specific primer was designed to include a portion of the coding sequence for verification

of the authenticity of the product by sequence comparison. Products were visualized and fractionated from a 2.0 % agarose gel stained with ethidium bromide and cloned into the sequencing vector pGEM-T Easy™ (Promega) as per the manufacturer's directions. Sequencing was performed using an ABI 310 and 377 automated sequencer. The reactions produced a number of fragments possibly depending on the number of transcription start sites and the integrity of the cDNA or the mRNA from which it was constructed. The sequences of the resulting RACE products and primers are found in appendix G. A schematic of the two longest clones (B and I) aligned to the 5' end of the Scn10a coding sequence is shown in figure 9.

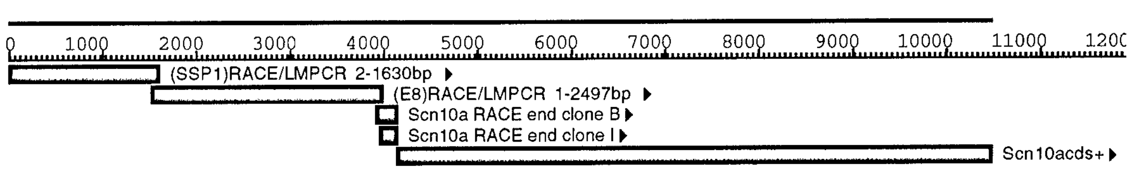
Figure 9: Alignment of the RACE products B and I to the 5' end of the Scn10a coding sequence



LM-PCR was performed with the use of the Genomewalking™ kit from Clontech. Primers (custom made by Gibco/BRL) were designed to extend into regions of genomic DNA in a 5' direction from the end of the Scn10a gene. The kit supplies genomic DNA cut with blunt end restriction enzymes to which an adaptor has been ligated. The kit supplies primers directed to that adaptor. The adaptor primers are used with gene specific primers in primary and nested PCR reactions. A polymerase mix consisting of *Tth* polymerase and a proofreading polymerase allow

for the high fidelity amplification of fragments up to 6 kb. PCR fragments from the library or libraries yielding a significant size and purity were further purified from agarose gels and cloned into a sequencing vector (pGEM-T easy™ vector (Promega)) as per the manufacturer's directions. Sequencing was performed using an ABI 310 and 377 automated sequencers. LM-PCR was performed outward from the first protein coding exon about 920 bp into an intron located upstream from this exon and from the upstream ends of the RACE reaction products about 4.0 kb into what we hope is the promoter region of the Scn10a gene. The resulting sequences and primers for the LM-PCR into the 5'UTR intron and the LM-PCR out from the RACE product sequences can be found in appendices H and I respectively. The 4.0 kb fragment was constructed in two steps as shown in figure 10 and is currently being cloned into the EGFP-N1 reporter vector (Clontech) for screening promoter activity. Figure 10 also shows orientation of the 4.0kb fragment with respect to the coding sequence and the 5' RACE products.

Figure 10: Schematic of RACE followed by 2 rounds of LM-PCR (upstream 5' UTR intron has been bypassed: LM-PCR = genomic sequence/RACE and Scn10a cds are mRNA based.)



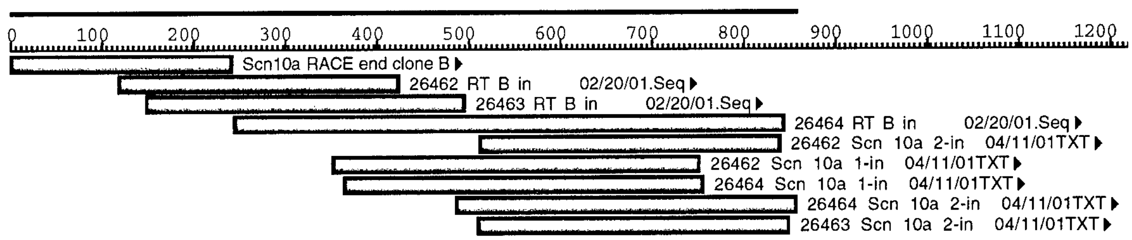
Genomic screening was performed through Incyte Genomics (Palo Alto, CA). The screening was of a mouse genomic library constructed in bacterial artificial chromosomes. The method of screening was PCR based, and primers were designed to the 5' coding sequence (first coding exon) from previously reported sequence information on the mouse genomic sequence (Souslova *et al.*). The screening process identified three unique BAC clones. Many introns have been identified in the mouse genomic Scn10a clone. Introns, generally, are regions of DNA sequence that interrupt the protein-coding portion of a gene. They are spliced out following transcription to yield a continuous coding mRNA. Coding exons on the 5' and 3' ends of the Scn10a gene were used as separate templates for secondary screening of the positives clones we received. The identification of clones yielding positive results for each primer set indicated the presence of the intact coding portion of the gene plus any introns in all three clones. The primers used to PCR screen the mouse genomic library and the sequence of the resulting PCR product are shown in appendix J.

Genomic sequencing was performed on the three clones upon arrival. Clones were supplied as glycerol stocks. The stocks were plated and individual clones were selected with the primers used for the screening protocol to ensure a homogeneous population for future work. We have found that extensive sequencing of the BAC clones is possible with only minor modifications to the protocol supplied with the Perkin Elmer Sequencing kit designed for the ABI 310 and 377 sequence analyzer. The modification involves an increase (10 fold) of DNA template concentration. Initial sequencing with primers directed toward distant regions of the gene such as the 3' and 5' ends were performed as mentioned above to establish the extent of the gene contained in the insert. We have also identified the ends of our RACE reaction and LM-PCR from the RACE reaction products in all three clones indicating that a fair amount of genomic sequence is present beyond the coding exons. Restriction mapping of the BAC clones is also currently underway using a combination of blotting and PCR. Figure 11 contains primer information (A) and alignments of the various regions upon which we have focused in characterizing how intact our BAC clones are with respect to the Scn10a gene. Figure 11 B and C shows sequencing into the 5'UTR intron from both downstream and upstream directions. Figure 11 D shows the direct sequencing of the 3' end of the coding sequence and 11 E shows the direct sequencing of the 5' end of the LM-PCR from RACE products. These figures combined show the presence of the entire coding region of the gene on our BAC clones as well as at least ~4.0 kb above the RACE ends or putative transcriptional start site.

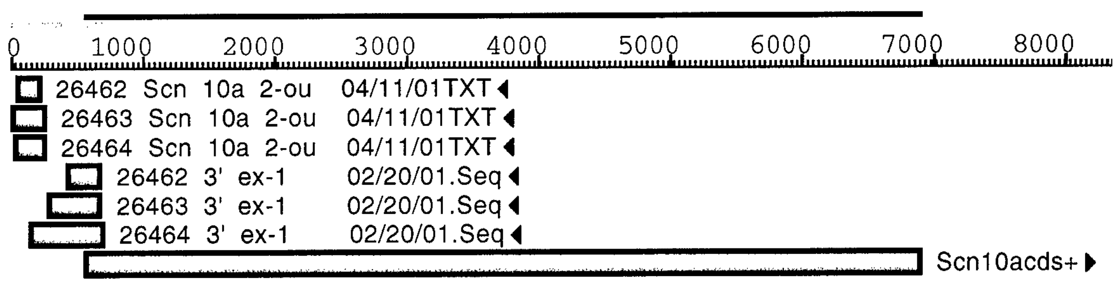
Figure 11: Direct sequencing of BAC clones 26462/26463/26464:

A: Primers:
GTGTAAGTTTCGCAGAGCTGGGGTC-RT B in
TCATGGACAAAGCGTAAGTGC- Scn10a Intron-in1
CCTGCATGCTCTACCAAGTCG- Scn10a Intron-in2
GGTGACAGCCTGACCACTGC- Scn10a Intron-out1
GCTTTGTAAGAAGCTCCATCC- Scn10a Intron-out2
CCTGTGTGTGCTGTAAAAAGGATC - 3' EX-1

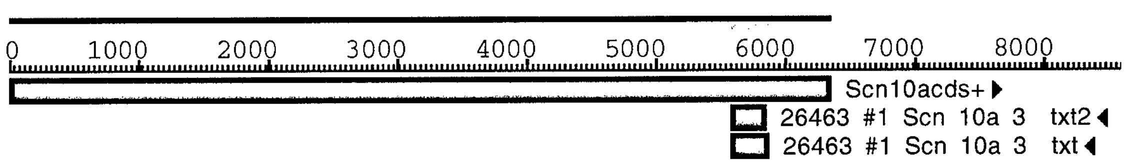
B: Sequencing direct in into 5'UTR intron



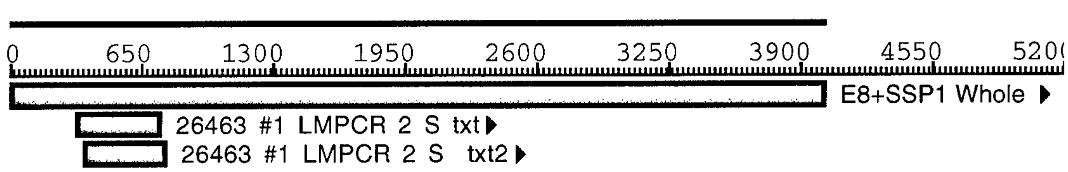
C: Sequencing direct out into 5'UTR intron



D: 3' end of coding sequence found in all three BAC clones (only BAC clone #26463 shown-sequenced twice txt and txt2)



E: 5' end of combined LM-PCR from RACE products (only BAC clone #26463 shown-sequenced twice txt and txt2)



Key Research Accomplishments

- **Cloning and sequencing of the mouse Scn10a cDNA**
- **Functional heterologous expression of mouse Scn10a cDNA in sympathetic neurons**
- **Biophysical characterization of sodium currents arising from Scn10a expression**
- **Cloning and sequencing of 4 Kbp of upstream genomic DNA containing putative promoter elements**
- **Identification of BAC (bacterial artificial chromosome) clones harboring the Scn10a gene**

Reportable Outcomes

Abstracts:

1. Ikeda, S.R., King, M.M., Aronstam, R.S. and Puhl, H.L. Cloning and expression of cDNA encoding a tetrodotoxin-resistant (TTX-R) sodium channel (Scn10a) from mouse dorsal root ganglion neurons. *Experimental Biology Meeting,* 2001.

2. Puhl, H.L., King, M.M., Aronstam, R.S. and Ikeda, S.R. Cloning and functional characterization of mouse cDNA encoding a tetrodotoxin-resistant (TTX-R) sodium channel (Scn10a). *Soc. Neurosci. Abstr.,* 2001.

Conclusions

a. A murine ortholog of the TTX-resistant sodium channel Scn10a (SNS/PN3) was cloned from mouse DRG neuron mRNA using the PCR.

b. The predicted protein sequence is highly homologous to the rat, dog, and human Scn10a gene product. Surprisingly, the nucleotide sequence deviates significantly from a published mouse genomic sequence perhaps suggesting a gene duplication.

c. Intranuclear injection of the cloned cDNA into rat sympathetic neurons results in robust TTX-R sodium currents with kinetics characteristic of TTX-R sodium currents recorded from mouse DRG neurons.

d. Steady-state inactivation and activation curves for the heterologously expressed Scn10a sodium current were shifted toward more positive potentials when compared with native DRG TTX-R sodium currents. This suggests that accessory proteins or post-translational modifications may be required to recapitulate the native phenotype.

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Appendix A
Mouse Scn10a cDNA nucleotide sequence

```
                10          20          30          40          50          60
ATG GAG TTC CCC TTT GGG TCC GTG GGA ACT ACC AAC TTC AGA CGG TTC ACT CCA GGG TCG
 M   E   F   P   F   G   S   V   G   T   T   N   F   R   R   F   T   P   G   S>

                70          80          90         100         110         120
CTG GCA GAG ATC GAG AAG CAG ATC GCT GCC CAC CGC GCC GCC AAG AAG GGC AGA CCT AAG
 L   A   E   I   E   K   Q   I   A   A   H   R   A   A   K   K   G   R   P   K>

               130         140         150         160         170         180
CAA AGA GGA CAG AAG GAC AAG AGT GAG AAG CCC AGG CCT CAG TTG GAC TTG AAG GCC TGT
 Q   R   G   Q   K   D   K   S   E   K   P   R   P   Q   L   D   L   K   A   C>

               190         200         210         220         230         240
AAC CAG CTG CCC AGG TTC TAT GGC GAG CTC CCA GCA GAG CTG GTC GGG GAG CCC CTG GAG
 N   Q   L   P   R   F   Y   G   E   L   P   A   E   L   V   G   E   P   L   E>

               250         260         270         280         290         300
GAC CTG GAT CCT TTC TAC AGC ACA CAC CGG ACA TTC ATA GTG TTG GAT AAA AGC AGG ACC
 D   L   D   P   F   Y   S   T   H   R   T   F   I   V   L   D   K   S   R   T>

               310         320         330         340         350         360
ATT TCC AGA TTC AGT GCC ACT TGG GCT CTG TGG CTC TTC AGT CCC TTC AAC CTG ATC AGA
 I   S   R   F   S   A   T   W   A   L   W   L   F   S   P   F   N   L   I   R>

               370         380         390         400         410         420
AGA ACA GCC ATC AAA GTG TCC GTC CAC TCC TGG TTC TCC ATA TTT ATC ACT GTC ACT ATT
 R   T   A   I   K   V   S   V   H   S   W   F   S   I   F   I   T   V   T   I>

               430         440         450         460         470         480
TTG GTC AAC TGT GTG TGC ATG ACC CGA ACT GAT CTT CCA GAG AAA CTC GAG TAT GCC TTC
 L   V   N   C   V   C   M   T   R   T   D   L   P   E   K   L   E   Y   A   F>

               490         500         510         520         530         540
ACT GTT GTT TAC ACC TTC GAG GCT CTG ATA AAG ATA CTG GCA AGA GGG TTT TGT CTA AAT
 T   V   V   Y   T   F   E   A   L   I   K   I   L   A   R   G   F   C   L   N>

               550         560         570         580         590         600
GAA TTC ACT TAT CTT CGA GAT CCC TGG AAC TGG CTG GAC TTC AGT GTC ATT ACC CTG GCG
 E   F   T   Y   L   R   D   P   W   N   W   L   D   F   S   V   I   T   L   A>

               610         620         630         640         650         660
TAT GTG GGT GCA GCG ATA GAC CTC CGA GGA ATC TCA GGC CTG CGG ACA TTC CGA GTT CTC
 Y   V   G   A   A   I   D   L   R   G   I   S   G   L   R   T   F   R   V   L>

               670         680         690         700         710         720
AGG GCC CTG AAG ACT GTT TCT GTG ATC CCA GGA CTG AAG GTC ATC GTG GGA GCC CTG ATC
 R   A   L   K   T   V   S   V   I   P   G   L   K   V   I   V   G   A   L   I>

               730         740         750         760         770         780
CAC TCA GTG AGG AAG CTG GCC GAC GTG ACC ATC CTC ACA GTC TTC TGC CTG AGT GTC TTT
 H   S   V   R   K   L   A   D   V   T   I   L   T   V   F   C   L   S   V   F>

               790         800         810         820         830         840
GCC TTG GTG GGC CTG CAG CTC TTC AAG GGG AAC CTC AAG AAT AAA TGC ATT AAG AAC GGC
 A   L   V   G   L   Q   L   F   K   G   N   L   K   N   K   C   I   K   N   G>

               850         860         870         880         890         900
ACA GAT CCG CAC AAG GCT GAC AAT CTC TCA TCT GAA ATG GCA GGA GAC ATC TTC ATC AAG
 T   D   P   H   K   A   D   N   L   S   S   E   M   A   G   D   I   F   I   K>

               910         920         930         940         950         960
CCC GGT ACT ACG GAT CCC TTG TTG TGT GGC AAT GGA TCT GAT GCT GGC CAC TGC CCT AAT
 P   G   T   T   D   P   L   L   C   G   N   G   S   D   A   G   H   C   P   N>

               970         980         990        1000        1010        1020
GAT TAT GTC TGC CGG AAA ACT TCT GAC AAC CCG GAT TTT AAC TAC ACC AGC TTT GAT TCC
 D   Y   V   C   R   K   T   S   D   N   P   D   F   N   Y   T   S   F   D   S>
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1030       1040       1050       1060       1070       1080
TTC GCG TGG GCG TTC CTC TCA CTG TTC CGT CTC ATG ACG CAG GAC TCC TGG GAA CGG CTG
 F   A   W   A   F   L   S   L   F   R   L   M   T   Q   D   S   W   E   R   L>

       1090       1100       1110       1120       1130       1140
TAC CAG CAG ACA CTC CGG GCT TCC GGG AAA ATG TAC ATG GTC TTT TTT GTG CTG GTC ATC
 Y   Q   Q   T   L   R   A   S   G   K   M   Y   M   V   F   F   V   L   V   I>

       1150       1160       1170       1180       1190       1200
TTC CTT GGA TCA TTC TAC CTG GTC AAT TTG ATC TTG GCT GTG GTC ACC ATG GCA TAT GAG
 F   L   G   S   F   Y   L   V   N   L   I   L   A   V   V   T   M   A   Y   E>

       1210       1220       1230       1240       1250       1260
GAA CAG AGC CAG GCA ACA ATT GCA GAA ATC GAA GCC AAG GAG AAG AAG TTC AAG GAA GCC
 E   Q   S   Q   A   T   I   A   E   I   E   A   K   E   K   K   F   K   E   A>

       1270       1280       1290       1300       1310       1320
CTC GAG GTG CTG CAG AAA GAA CAG GAG GTG CTG GCA GCG CTG GGC ATT GGC ACA ACC TCG
 L   E   V   L   Q   K   E   Q   E   V   L   A   A   L   G   I   G   T   T   S>

       1330       1340       1350       1360       1370       1380
CTC TAT TCC CAC AAC GGA TCA CCC TTA GCC CCC AAA AAC GCC AAT GAG AGA AGA CCC AGG
 L   Y   S   H   N   G   S   P   L   A   P   K   N   A   N   E   R   R   P   R>

       1390       1400       1410       1420       1430       1440
GTG AAA TCA AGG ATG TCA GAA GGC TCG ACA GAT GAC AAC AGA TCA CTA CAA TCC GAC CCT
 V   K   S   R   M   S   E   G   S   T   D   D   N   R   S   L   Q   S   D   P>

       1450       1460       1470       1480       1490       1500
TAC AAC CAG CGC AGG ATG TCT TTC CTA GGC CTT TCT TCT GGA AGA CGC AGG GCT AGC CAC
 Y   N   Q   R   R   M   S   F   L   G   L   S   S   G   R   R   R   A   S   H>

       1510       1520       1530       1540       1550       1560
AGC AGT GTG TTC CAC TTC CGA GCA CCC AGC CAA GAC GTC TCA TTT CCT GAT GGG ATC TTG
 S   S   V   F   H   F   R   A   P   S   Q   D   V   S   F   P   D   G   I   L>

       1570       1580       1590       1600       1610       1620
GAT GAC GGG GTC TTT CAT GGA GAT CAG GAA AGC CGT CGA AGT TCC ATA TTG CTG GGC AGG
 D   D   G   V   F   H   G   D   Q   E   S   R   R   S   S   I   L   L   G   R>

       1630       1640       1650       1660       1670       1680
GGT GCC GGG CAG GCA GGT CCT CTC CCC AGG AGT CCA CTG CCT CAG TCC CCC AAC CCT GGC
 G   A   G   Q   A   G   P   L   P   R   S   P   L   P   Q   S   P   N   P   G>

       1690       1700       1710       1720       1730       1740
CCT AGA CGT GGA GAA GAG GGA CAG CGT GGA GTG CCC ACT GGT GAG CTT GCC ACT GGA GCG
 P   R   R   G   E   E   G   Q   R   G   V   P   T   G   E   L   A   T   G   A>

       1750       1760       1770       1780       1790       1800
CCT GAA GGC CCG GCA CTC GAT GCT GCA GGA CAG AAG AAC TTC CTG TCT GCA GAC TAC TTG
 P   E   G   P   A   L   D   A   A   G   Q   K   N   F   L   S   A   D   Y   L>

       1810       1820       1830       1840       1850       1860
AAT GAA CCT TTC CGA GCA CAG AGG GCA ATG AGT GTT GTC AGT ATT ATG ACT TCT GTC ATT
 N   E   P   F   R   A   Q   R   A   M   S   V   V   S   I   M   T   S   V   I>

       1870       1880       1890       1900       1910       1920
GAG GAG CTG GAA GAG TCT AAG CTG AAG TGC CCA CCC TGC TTG ATC AGC TTA GCC CAG AAG
 E   E   L   E   E   S   K   L   K   C   P   P   C   L   I   S   L   A   Q   K>

       1930       1940       1950       1960       1970       1980
TAC CTG ATA TGG GAG TGC TGC CCC AAG TGG AAG AAA TTC AAG ATG GTG CTC TTC GAG CTG
 Y   L   I   W   E   C   C   P   K   W   K   K   F   K   M   V   L   F   E   L>

       1990       2000       2010       2020       2030       2040
GTG ACT GAC CCC TTC GCA GAG CTC ACC ATC ACC CTG TGC ATT GTG GTG AAT ACC GTC TTC
 V   T   D   P   F   A   E   L   T   I   T   L   C   I   V   V   N   T   V   F>

       2050       2060       2070       2080       2090       2100
ATG GCC ATG GAA CAC TAC CCC ATG ACT GAT GCC TTC GAT GCC ATG CTC CAA GCC GGC AAC
 M   A   M   E   H   Y   P   M   T   D   A   F   D   A   M   L   Q   A   G   N>
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        2110        2120        2130        2140        2150        2160
ATT GTC TTT ACT GTG TTT TTT ACA ATG GAG ATG GCC TTC AAG ATC ATT GCC TTC GAC CCC
 I   V   F   T   V   F   F   T   M   E   M   A   F   K   I   I   A   F   D   P>

        2170        2180        2190        2200        2210        2220
TAC TAC TAC TTC CAG AAG AAG TGG AAC ATC TTC GAC TGT GTC ATC GTC ACC GTG AGC CTG
 Y   Y   Y   F   Q   K   K   W   N   I   F   D   C   V   I   V   T   V   S   L>

        2230        2240        2250        2260        2270        2280
CTG GAG CTG AGC ACA TCC AAG AAG GGC AGC TTG TCT GTG CTC CGC ACC TTC CGC TTG CTT
 L   E   L   S   T   S   K   K   G   S   L   S   V   L   R   T   F   R   L   L>

        2290        2300        2310        2320        2330        2340
CGG GTC TTC AAG CTG GCC AAG TCC TGG CCC ACC CTG AAC ATG CTC ATC AAG ATC ATC GGG
 R   V   F   K   L   A   K   S   W   P   T   L   N   M   L   I   K   I   I   G>

        2350        2360        2370        2380        2390        2400
AAC TCT GTG GGG GCC CTG GGC AAC CTG ACC TTC ATC CTG GCC ATC ATC GTC TTT ATC TTC
 N   S   V   G   A   L   G   N   L   T   F   I   L   A   I   I   V   F   I   F>

        2410        2420        2430        2440        2450        2460
GCC CTG GTG GGA AAG CAG CTC CTC TCA GAG AAC TAT GGG TGC CGC AGG GAT GGC ATC TCC
 A   L   V   G   K   Q   L   L   S   E   N   Y   G   C   R   R   D   G   I   S>

        2470        2480        2490        2500        2510        2520
GTG TGG AAT GGT GAG AGG CTG CGC TGG CAC ATG TGT GAC TTC TTC CAT TCC TTC CTC GTC
 V   W   N   G   E   R   L   R   W   H   M   C   D   F   F   H   S   F   L   V>

        2530        2540        2550        2560        2570        2580
GTC TTC CGG ATC CTC TGC GGG GAG TGG ATC GAG AAC ATG TGG GTC TGC ATG GAG GTC AGC
 V   F   R   I   L   C   G   E   W   I   E   N   M   W   V   C   M   E   V   S>

        2590        2600        2610        2620        2630        2640
CAG GAC TAC ATC TGC CTC ACC CTC TTC TTG ACA GTG ATG GTG CTA GGC AAC CTG GTG GTG
 Q   D   Y   I   C   L   T   L   F   L   T   V   M   V   L   G   N   L   V   V>

        2650        2660        2670        2680        2690        2700
CTC AAC CTA TTC ATC GCT TTA CTG CTG AAC TCC TTC AGT GCG GAC AAC CTC ACA GCC CCA
 L   N   L   F   I   A   L   L   L   N   S   F   S   A   D   N   L   T   A   P>

        2710        2720        2730        2740        2750        2760
GAG GAT GAC GGG GAG GTG AAC AAC TTG CAG GTA GCA CTG GCC CGG ATT CAG GTA TTT GGC
 E   D   D   G   E   V   N   N   L   Q   V   A   L   A   R   I   Q   V   F   G>

        2770        2780        2790        2800        2810        2820
CAT CGG GCC AGT CGG GCC ATT ACC AGT TAC ATC AGA AGC CAC TGC CGG CTC CGC TGG CCC
 H   R   A   S   R   A   I   T   S   Y   I   R   S   H   C   R   L   R   W   P>

        2830        2840        2850        2860        2870        2880
AAG GTG GAG ACC CAG CTG GGG ATG AAA CCC CCA CTC ACC AGC TGC AAA GCT GAG AAC CAC
 K   V   E   T   Q   L   G   M   K   P   P   L   T   S   C   K   A   E   N   H>

        2890        2900        2910        2920        2930        2940
ATT GCT ACT GAT GCT GNC AAT GCT GCA GTG GGG AAC CAG GCA AAG CCA GCT CTT GGT GGC
 I   A   T   D   A   X   N   A   A   V   G   N   Q   A   K   P   A   L   G   G>

        2950        2960        2970        2980        2990        3000
CCC AAG GAG AAC CAC GGG GAC TTC ATC ACT GAT CCT AAC GTG TGG GTC TCT GTG CCC ATT
 P   K   E   N   H   G   D   F   I   T   D   P   N   V   W   V   S   V   P   I>

        3010        3020        3030        3040        3050        3060
GCT GAG GGG GAG TCC GAC CTT GAT GAG CTC GAG GAA GAT GTG GAG CAT GCT TCT CAG AGC
 A   E   G   E   S   D   L   D   E   L   E   E   D   V   E   H   A   S   Q   S>

        3070        3080        3090        3100        3110        3120
TCC TGG CAG GAA GAG AGC CCC AAA GGG CAG GAG CTG CTG CAG CAA GTC CAA AAG TGT GAA
 S   W   Q   E   E   S   P   K   G   Q   E   L   L   Q   Q   V   Q   K   C   E>

        3130        3140        3150        3160        3170        3180
GAT CAC CAG GCA GCC CGA AGC CCA CCC TCC GGG ATG TCC TCT GAG GAC CTG GCT CCA TAC
 D   H   Q   A   A   R   S   P   P   S   G   M   S   S   E   D   L   A   P   Y>
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         3190        3200        3210        3220        3230        3240
CTG GGG GAG AGA TGG CAG AGG GAG GAG AGC CCT CGG GTC CCT GCC GAG GGA GTG GAT GAC
 L   G   E   R   W   Q   R   E   E   S   P   R   V   P   A   E   G   V   D   D>

         3250        3260        3270        3280        3290        3300
ACA AGC TCC TCC GAG GGC AGC ACG GTG GAC TGC CCG GAC CCA GAG GAG ATC CTG AGG AAG
 T   S   S   S   E   G   S   T   V   D   C   P   D   P   E   E   I   L   R   K>

         3310        3320        3330        3340        3350        3360
ATC CCT GAG CTG GCG GAG GAG CTG GAC GAG CCC GAT GAC TGT TTC CCA GAA GGC TGC ACT
 I   P   E   L   A   E   E   L   D   E   P   D   D   C   F   P   E   G   C   T>

         3370        3380        3390        3400        3410        3420
CGC CGC TGT CCC TGC TGC AAA GTG AAC ACC AGT AAG TTT CCT TGG GCC ACG GGC TGG CAG
 R   R   C   P   C   C   K   V   N   T   S   K   F   P   W   A   T   G   W   Q>

         3430        3440        3450        3460        3470        3480
GTG CGC AAA ACC TGT TAC CGC ATT GTG GAG CAC AGC TGG TTT GAG AGT TTC ATT ATC TTC
 V   R   K   T   C   Y   R   I   V   E   H   S   W   F   E   S   F   I   I   F>

         3490        3500        3510        3520        3530        3540
ATG ATC CTG CTC AGC AGT GGA GCG CTG GCC TTT GAG GAT AAC TAC CTG GAA GAA AAG CCC
 M   I   L   L   S   S   G   A   L   A   F   E   D   N   Y   L   E   E   K   P>

         3550        3560        3570        3580        3590        3600
CGA GTG AAG TCT GTG CTG GAG TAC ACT GAC AGA GTG TTC ACT TTC ATC TTT GTA TTC GAG
 R   V   K   S   V   L   E   Y   T   D   R   V   F   T   F   I   F   V   F   E>

         3610        3620        3630        3640        3650        3660
ATG TTG CTC AAG TGG GTA GCT TAT GGC TTC AAA AAA TAT TTC ACC AAT GCC TGG TGC TGG
 M   L   L   K   W   V   A   Y   G   F   K   K   Y   F   T   N   A   W   C   W>

         3670        3680        3690        3700        3710        3720
CTG GAC TTC CTC ATC GTG AAT ATC TCC CTC ACA AGC CTC ATA GCC AAG ATC CTC GAG TAT
 L   D   F   L   I   V   N   I   S   L   T   S   L   I   A   K   I   L   E   Y>

         3730        3740        3750        3760        3770        3780
TCA GAC GTG GCG TCC ATC AAA GCC CTT CGG ACT CTC CGT GCC CTC CGG CCG CTG CGG GCT
 S   D   V   A   S   I   K   A   L   R   T   L   R   A   L   R   P   L   R   A>

         3790        3800        3810        3820        3830        3840
CTG TCT CGA TTC GAA GGC ATG AGG GTA GTG GTG GAT GCC TTG GTG GGC GCC ATT CCC TCC
 L   S   R   F   E   G   M   R   V   V   V   D   A   L   V   G   A   I   P   S>

         3850        3860        3870        3880        3890        3900
ATC ATG AAC GTC CTC CTC GTC TGC CTC ATC TTC TGG CTC ATC TTC AGC ATC ATG GGT GTG
 I   M   N   V   L   L   V   C   L   I   F   W   L   I   F   S   I   M   G   V>

         3910        3920        3930        3940        3950        3960
AAC CTC TTC GCC GGG AAA TTT TCG AGA TGT GTC GAC ACC AGA AGC AAC CCA TTT TCC GTC
 N   L   F   A   G   K   F   S   R   C   V   D   T   R   S   N   P   F   S   V>

         3970        3980        3990        4000        4010        4020
GTG AAT TCG ACA TTC GTG ACT AAC AAG TCT GAC TGT TAC AAT CAA AAC AAT ACT GGC CAC
 V   N   S   T   F   V   T   N   K   S   D   C   Y   N   Q   N   N   T   G   H>

         4030        4040        4050        4060        4070        4080
TTC TTC TGG GTT AAC GTC AAA GTC AAC TTC GAC AAC GTT GCT ATG GGC TAC CTC GCG CTT
 F   F   W   V   N   V   K   V   N   F   D   N   V   A   M   G   Y   L   A   L>

         4090        4100        4110        4120        4130        4140
CTC CAG GTG GCA ACC TTC AAA GGC TGG ATG GAC ATT ATG TAT GCA GCT GTC GAT TCT CGA
 L   Q   V   A   T   F   K   G   W   M   D   I   M   Y   A   A   V   D   S   R>

         4150        4160        4170        4180        4190        4200
GAT ATC AAC AGT CAG CCC AAT TGG GAG GAG AGC CTG TAC ATG TAC CTA TAC TTC GTC GTC
 D   I   N   S   Q   P   N   W   E   E   S   L   Y   M   Y   L   Y   F   V   V>

         4210        4220        4230        4240        4250        4260
TTC ATC ATT TTC GGT GGC TTC TTC ACG CTG AAT CTC TTT GTC GGG GTC ATC ATT GAC AAC
 F   I   I   F   G   G   F   F   T   L   N   L   F   V   G   V   I   I   D   N>
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4270 4280 4290 4300 4310 4320
TTC AAT CAA CAG AAA AAA AAG CTA GGG GGC CAG GAC ATC TTC ATG ACA GAG GAG CAG AAG
F N Q Q K K K L G G Q D I F M T E E Q K>

4330 4340 4350 4360 4370 4380
AAG TAC TAC AAT GCC ATG AAG AAG CTG GGC TCC AAG AAA CCC CAG AAG CCC ATC CCA CGG
K Y Y N A M K K L G S K K P Q K P I P R>

4390 4400 4410 4420 4430 4440
CCT TTG AAT AAG TAC CAG GGC TTC GTG TTT GAC ATT GTG ACC AGG CAA GCA TTT GAC ATC
P L N K Y Q G F V F D I V T R Q A F D I>

4450 4460 4470 4480 4490 4500
ATC ATC ATG GCT CTC ATC TGC CTC AAC ATG ATC ACC ATG ATG GTG GAG ACC GAC AAT CAG
I I M A L I C L N M I T M M V E T D N Q>

4510 4520 4530 4540 4550 4560
AGC GAG GAG AAG ACG AAG GTC CTG GGC AGA ATC AAC CAG TTC TTC GTG GCC GTC TTC ACG
S E E K T K V L G R I N Q F F V A V F T>

4570 4580 4590 4600 4610 4620
GGC GAG TGT GTG ATG AAG ATG TTC GCC CTT CGG CAG TAT TAC TTC ACC AAC GGC TGG AAT
G E C V M K M F A L R Q Y Y F T N G W N>

4630 4640 4650 4660 4670 4680
GTG TTC GAC TTC ATT GTG GTG ATT CTG TCC ATT TCT AGT CTG TTG TTT TCT GCG ATC CTT
V F D F I V V I L S I S S L L F S A I L>

4690 4700 4710 4720 4730 4740
AGC TCA CTA GAA AGT TAC TTC TCC CCC ACG CTC TTA CGC GTC ATC CGT CTG GCC AGG ATC
S S L E S Y F S P T L L R V I R L A R I>

4750 4760 4770 4780 4790 4800
GGC CGC ATC CTC AGG CTG ATT CGA GCA GCC AAG GGG ATT CGC ACG CTG CTC TTC GCC CTC
G R I L R L I R A A K G I R T L L F A L>

4810 4820 4830 4840 4850 4860
ATG ATG TCC CTG CCC GCC CTC TTC AAC ATC GGC CTC CTC CTC TTC CTC GTC ATG TTC ATC
M M S L P A L F N I G L L L F L V M F I>

4870 4880 4890 4900 4910 4920
TAC TCC ATC TTC GGC ATG ACC AGC TTC GCT AAT GTC ATA GAT GAG GCT GGC ATC GAC GAC
Y S I F G M T S F A N V I D E A G I D D>

4930 4940 4950 4960 4970 4980
ATG TTC AAC TTC AAG ACC TTT GGC AAC AGC ATG CTG TGC CTT TTC CAG ATC ACC ACG TCG
M F N F K T F G N S M L C L F Q I T T S>

4990 5000 5010 5020 5030 5040
GCT GGC TGG GAT GGC CTC CTC AGC CCC ATC CTC AAC ACA GGA CCC CCC TAC TGC GAC CCC
A G W D G L L S P I L N T G P P Y C D P>

5050 5060 5070 5080 5090 5100
AAC CGG CCC AAC AGC AAT GGC TCC AAG GGG AAT TGT GGA AGC CCA GCG GTG GGC ATC CTC
N R P N S N G S K G N C G S P A V G I L>

5110 5120 5130 5140 5150 5160
TTC TTC ACC ACC TAC ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATT GCA GTG
F F T T Y I I I S F L I V V N M Y I A V>

5170 5180 5190 5200 5210 5220
ATT CTG GAG AAC TTC AAT GTG GCC ACA GAA GAG AGC ACG GAG CCC CTG AGC GAG GAC GAC
I L E N F N V A T E E S T E P L S E D D>

5230 5240 5250 5260 5270 5280
TTT GAC ATG TTC TAT GAG ACC TGG GAG AAG TTT GAC CCG GAG GCC ACC CAG TTC ATT GCC
F D M F Y E T W E K F D P E A T Q F I A>

5290 5300 5310 5320 5330 5340
TTT TCT GCC CTC TCA GAC TTT GCA GAC ACA CTC TCT GGC CCT CTT AGA ATC CCA AAA CCT
F S A L S D F A D T L S G P L R I P K P>

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         5350        5360        5370         5380        5390        5400
AAT CAG AAT ATA TTA ATC CAG ATG GAC CTG CCG TTG GTC CCC GGA GAT AAG ATC CAC TGT
 N   Q   N   I   L   I   Q   M   D   L   P   L   V   P   G   D   K   I   H   C>

         5410        5420        5430         5440        5450        5460
TTG GAC ATC CTC TTT GCC TTC ACA AAG AAT GTC TTG GGA GAA TCT GGG GAG TTG GAT TCT
 L   D   I   L   F   A   F   T   K   N   V   L   G   E   S   G   E   L   D   S>

         5470        5480        5490         5500        5510        5520
CTG AAG ACT AAT ATG GAA GAG AAG TTT ATG GCA ACT AAT CTT TCC AAA GCA TCC TAT GAA
 L   K   T   N   M   E   E   K   F   M   A   T   N   L   S   K   A   S   Y   E>

         5530        5540        5550         5560        5570        5580
CCA ATA GCA ACC ACC CTC CGG TGC AAG CAG GAA GAC ATC TCA GCC ACC ATT ATT CAA AAG
 P   I   A   T   T   L   R   C   K   Q   E   D   I   S   A   T   I   I   Q   K>

         5590        5600        5610         5620        5630        5640
GCC TAT CGG AAC TAC ATG TTG CAA CGC TCC TTG ATG CTC TCC AAC CCC CTG CAT GTG CCC
 A   Y   R   N   Y   M   L   Q   R   S   L   M   L   S   N   P   L   H   V   P>

         5650        5660        5670         5680        5690        5700
AGG GCT GAG GAG GAT GGC GTG TCA CTC CCC AGG GAA GGC TAT GTT ACA TTC ATG GCA AAT
 R   A   E   E   D   G   V   S   L   P   R   E   G   Y   V   T   F   M   A   N>

         5710        5720        5730         5740        5750        5760
GAC AAC GGT GGG CTC CCA GAC AAA TCG GAA ACT GCT TCT GCT ACG TCT TTC CCA CCA TCC
 D   N   G   G   L   P   D   K   S   E   T   A   S   A   T   S   F   P   P   S>

         5770        5780        5790         5800        5810        5820
TAT GAC AGC GTC ACC AGG GGC CTG AGT GAC AGG GCC AAC ATT AGC ACA TCT AGC TCA ATG
 Y   D   S   V   T   R   G   L   S   D   R   A   N   I   S   T   S   S   S   M>

         5830        5840        5850         5860        5870
CAA AAT GAA GAT GAA GTC ACT GCT AAG GAA GGG AAG AGC CCT GGA CCT CAG TGA
 Q   N   E   D   E   V   T   A   K   E   G   K   S   P   G   P   Q   *>
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Appendix B

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                                 10        20        30        40
Y09108-cds            ATGGAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCA
mScn10a cds (GRI)     ATGGAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCA

                                 50        60        70        80
Y09108-cds            GACGGTTCACTCCAGAGTCGCTGGCAGAGATCGAGAAGCA
mScn10a cds (GRI)     GACGGTTCACTCCAGGGTCGCTGGCAGAGATCGAGAAGCA

                                 90       100       110       120
Y09108-cds            GATCGCTGCCCACCGCGCCGCCAAGAAGGGCAGAACTAAG
mScn10a cds (GRI)     GATCGCTGCCCACCGCGCCGCCAAGAAGGGCAGACCTAAG

                                130       140       150       160
Y09108-cds            CAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTC
mScn10a cds (GRI)     CAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTC

                                170       180       190       200
Y09108-cds            AGTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTCTA
mScn10a cds (GRI)     AGTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTCTA

                                210       220       230       240
Y09108-cds            TGGCGAGCTCCCAGCAGAGCTGGTCGGGGAGCCCCTGGAG
mScn10a cds (GRI)     TGGCGAGCTCCCAGCAGAGCTGGTCGGGGAGCCCCTGGAG

                                250       260       270       280
Y09108-cds            GACCTGGATCCTTTTTACAGCACACACAGGACATTCATAC
mScn10a cds (GRI)     GACCTGGATCCTTTCTACAGCACACACCGGACATTCATAG

                                290       300       310       320
Y09108-cds            TGTTGAATAAAAGCAGGACCATTTCCAGATTCAGTGCCAC
mScn10a cds (GRI)     TGTTGGATAAAAGCAGGACCATTTCCAGATTCAGTGCCAC

                                330       340       350       360
Y09108-cds            TTGGGCTCTGTGGCTCTTCAGTCCCTTCAACCTGATCAGA
mScn10a cds (GRI)     TTGGGCTCTGTGGCTCTTCAGTCCCTTCAACCTGATCAGA

                                370       380       390       400
Y09108-cds            AGAACAGCCATCAAAGTGTCCGTCCACTCCTGGTTCTCCA
mScn10a cds (GRI)     AGAACAGCCATCAAAGTGTCCGTCCACTCCTGGTTCTCCA

                                410       420       430       440
Y09108-cds            TATTTATCACTGTCACTATTTTGGTCAACTGTGTGTGCAT
mScn10a cds (GRI)     TATTTATCACTGTCACTATTTTGGTCAACTGTGTGTGCAT
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                         490        500        510        520
Y09108-cds          ACTGTTGTTTACACCTTCGAGGCTCTGATAAAGATACTGG
mScn10a cds (GRI)   ACTGTTGTTTACACCTTCGAGGCTCTGATAAAGATACTGG

                         530        540        550        560
Y09108-cds          CAAGAGGGTTTTGTCT T AATGAATTCACTTATCTTCGAGA
mScn10a cds (GRI)   CAAGAGGGTTTTGTCT A AATGAATTCACTTATCTTCGAGA

                         570        580        590        600
Y09108-cds          TCCCTGGAACTGGCTGGACTTCAGTGTCATTACC T TGGC A
mScn10a cds (GRI)   TCCCTGGAACTGGCTGGACTTCAGTGTCATTACC C TGGC G

                         610        620        630        640
Y09108-cds          TA C GTGGGTGCAGCG G TAGACCTCCGAGGAATCTCAGGCC
mScn10a cds (GRI)   TA T GTGGGTGCAGCG A TAGACCTCCGAGGAATCTCAGGCC

                         650        660        670        680
Y09108-cds          TGCGGACATTCCGAGTTCTCAGGGCCCTGAAGACTGTTTC
mScn10a cds (GRI)   TGCGGACATTCCGAGTTCTCAGGGCCCTGAAGACTGTTTC

                         690        700        710        720
Y09108-cds          TGTGATCCCAGGACTGAAGGTCATCGTGGGAGCCCTGATC
mScn10a cds (GRI)   TGTGATCCCAGGACTGAAGGTCATCGTGGGAGCCCTGATC

                         730        740        750        760
Y09108-cds          CACTCAGTGAGGAAGCTGGCCGACGTGACCATCCTCACAG
mScn10a cds (GRI)   CACTCAGTGAGGAAGCTGGCCGACGTGACCATCCTCACAG

                         770        780        790        800
Y09108-cds          TCTTCTGCCTGAGTGTCTTTGCCTTGGTGGGCCTGCAGCT
mScn10a cds (GRI)   TCTTCTGCCTGAGTGTCTTTGCCTTGGTGGGCCTGCAGCT

                         810        820        830        840
Y09108-cds          CTTCAAGGGGAACCTCAAGAATAAATGCATTAAGAACGGC
mScn10a cds (GRI)   CTTCAAGGGGAACCTCAAGAATAAATGCATTAAGAACGGC

                         850        860        870        880
Y09108-cds          ACAGATCCGCACAAGGCTGACAATCTCTCATCTGAAATGG
mScn10a cds (GRI)   ACAGATCCGCACAAGGCTGACAATCTCTCATCTGAAATGG

                         890        900        910        920
Y09108-cds          CAG A AGACATCTTCATCAAGCCCGGTACTACGGATCCCTT
mScn10a cds (GRI)   CAG G AGACATCTTCATCAAGCCCGGTACTACGGATCCCTT

                         930        940        950        960
Y09108-cds          GTTGTGTGGCAATGG G TCTGATGCTGGCCACTGCCCTAAT
mScn10a cds (GRI)   GTTGTGTGGCAATGG A TCTGATGCTGGCCACTGCCCTAAT
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                        1010      1020      1030      1040
Y09108-cds          ACTACACCAGCTTTGATTCCTTTGCGTGGGCGTTCCTCTC
mScn10a cds (GRI)   ACTACACCAGCTTTGATTCCTTCGCGTGGGCGTTCCTCTC

                        1050      1060      1070      1080
Y09108-cds          ACTGTTCCGTCTCATGACGCAGGACTCCTGGGAACGGCTG
mScn10a cds (GRI)   ACTGTTCCGTCTCATGACGCAGGACTCCTGGGAACGGCTG

                        1090      1100      1110      1120
Y09108-cds          TACCAGCAGACACTCCGGGCTTCCGGGAAAATGTACATGG
mScn10a cds (GRI)   TACCAGCAGACACTCCGGGCTTCCGGGAAAATGTACATGG

                        1130      1140      1150      1160
Y09108-cds          TCTTTTTTGTGCTGGTCATTTTCCTTGGATCATTCTACCT
mScn10a cds (GRI)   TCTTTTTTGTGCTGGTCATCTTCCTTGGATCATTCTACCT

                        1170      1180      1190      1200
Y09108-cds          GGTCAATTTGATCTTGGCTGTGGTCACCATGGCATATGAG
mScn10a cds (GRI)   GGTCAATTTGATCTTGGCTGTGGTCACCATGGCATATGAG

                        1210      1220      1230      1240
Y09108-cds          GAACAGAGCCAGGCAACAATTGCAGAAATCGAAGCCAAGG
mScn10a cds (GRI)   GAACAGAGCCAGGCAACAATTGCAGAAATCGAAGCCAAGG

                        1250      1260      1270      1280
Y09108-cds          AAAAAAAGTTCCAGGAAGCCCTCGAGGTGCTGCAAAAAGA
mScn10a cds (GRI)   AGAAGAAGTTCAAGGAAGCCCTCGAGGTGCTGCAGAAAGA

                        1290      1300      1310      1320
Y09108-cds          ACAGGAGGTGTTGGCAGCGCTGGGAATTGACACAACTTCG
mScn10a cds (GRI)   ACAGGAGGTGCTGGCAGCGCTGGGCATTGGCACAACCTCG

                        1330      1340      1350      1360
Y09108-cds          TTTTATTCCCACAGCGGATCACCCTTAGCCTCCAAAAACG
mScn10a cds (GRI)   CTCTATTCCCACAACGGATCACCCTTAGCCCCCAAAAACG

                        1370      1380      1390      1400
Y09108-cds          CCAATGAGAGAAGACCCAGGGTGAAATCAAGGGTGTCAGA
mScn10a cds (GRI)   CCAATGAGAGAAGACCCAGGGTGAAATCAAGGATGTCAGA

                        1410      1420      1430      1440
Y09108-cds          AGGCTCCACAGATGACAACAGATCACCACAATCCGACCCT
mScn10a cds (GRI)   AGGCTCGACAGATGACAACAGATCACTACAATCCGACCCT

                        1450      1460      1470      1480
Y09108-cds          TACAACCAGCGCAGGATGTCCTTCCTAGGCCTTTCTTCTG
mScn10a cds (GRI)   TACAACCAGCGCAGGATGTCTTTCCTAGGCCTTTCTTCTG
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                    1530      1540      1550      1560
Y09108-cds          AGCACCCAGCCAAGACGTCTCATTTCCTGATGGGATCTTG
mScn10a cds (GRI)   AGCACCCAGCCAAGACGTCTCATTTCCTGATGGGATCTTG

                    1570      1580      1590      1600
Y09108-cds          GACGATGGGGTCTTTCATGGAGATCAGGAAAGCCGTCGAA
mScn10a cds (GRI)   GATGACGGGGTCTTTCATGGAGATCAGGAAAGCCGTCGAA

                    1610      1620      1630      1640
Y09108-cds          ATTCCATATTGCTGGGCAGGGGTGCCGGCCAGGCAGGTCC
mScn10a cds (GRI)   GTTCCATATTGCTGGGCAGGGGTGCCGGGCAGGCAGGTCC

                    1650      1660      1670      1680
Y09108-cds          TCTCCCCAGAAGTCCACTGCCTCAGTCCCCCAACCCTGGC
mScn10a cds (GRI)   TCTCCCCAGGAGTCCACTGCCTCAGTCCCCCAACCCTGGC

                    1690      1700      1710      1720
Y09108-cds          CGTAAACATGGAAAAGAGGGACAGCTTGGAATGCCCACTG
mScn10a cds (GRI)   CCTAGACGTGGAGAAGAGGGACAGCGTGGAGTGCCCACTG

                    1730      1740      1750      1760
Y09108-cds          GTGAACTTGCCGCTGGAACGCCTGAAGGCCCGGCACTCGA
mScn10a cds (GRI)   GTGAGCTTGCCACTGGAGCGCCTGAAGGCCCGGCACTCGA

                    1770      1780      1790      1800
Y09108-cds          TGCTGCAGGACAGAAGAACTTCCTGTCTGCAGGCTACTTG
mScn10a cds (GRI)   TGCTGCAGGACAGAAGAACTTCCTGTCTGCAGACTACTTG

                    1810      1820      1830      1840
Y09108-cds          AATGAACCTTTCCGAGCACAGAGGGCAATGAGTGTTGTCA
mScn10a cds (GRI)   AATGAACCTTTCCGAGCACAGAGGGCAATGAGTGTTGTCA

                    1850      1860      1870      1880
Y09108-cds          GTATCATGACTTCTGTCATTGAGGAACTGGAAGAATCTAA
mScn10a cds (GRI)   GTATTATGACTTCTGTCATTGAGGAGCTGGAAGAGTCTAA

                    1890      1900      1910      1920
Y09108-cds          GCTGAAGTGCCCACCCTGCTTGATCAGCTTCGCCCAAAAA
mScn10a cds (GRI)   GCTGAAGTGCCCACCCTGCTTGATCAGCTTAGCCCAGAAG

                    1930      1940      1950      1960
Y09108-cds          TATCTGATATGGGAATGCTGCCCCAAGTGGAGAAAATTCA
mScn10a cds (GRI)   TACCTGATATGGGAGTGCTGCCCCAAGTGGAAGAAATTCA

                    1970      1980      1990      2000
Y09108-cds          AAATGGTGCTCCTCGAACTGGTGACTGACCCCTTCGCAGA
mScn10a cds (GRI)   AGATGGTGCTCTTCGAGCTGGTGACTGACCCCTTCGCAGA
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                                2050                2060                2070                2080
Y09108-cds          A T G G C C A T G G A A C A C T A C C C C A T G A C T G A T G C T T T C G A T G
mScn10a cds (GRI)   A T G G C C A T G G A A C A C T A C C C C A T G A C T G A T G C C T T C G A T G

                                2090                2100                2110                2120
Y09108-cds          C C A T G C T C C A A G C C G G C A A C A T T G T C T T C A C T G T G T T T T T
mScn10a cds (GRI)   C C A T G C T C C A A G C C G G C A A C A T T G T C T T T A C T G T G T T T T T

                                2130                2140                2150                2160
Y09108-cds          T A C A A T G G A G A T G G C C T T C A A G A T C A T T G C T T T C G A C C C G
mScn10a cds (GRI)   T A C A A T G G A G A T G G C C T T C A A G A T C A T T G C C T T C G A C C C C

                                2170                2180                2190                2200
Y09108-cds          T A C T A C T A C T T C C A G A A G A A G T G G A A C A T C T T C G A C T G T G
mScn10a cds (GRI)   T A C T A C T A C T T C C A G A A G A A G T G G A A C A T C T T C G A C T G T G

                                2210                2220                2230                2240
Y09108-cds          T C A T T G T C A C C G T G A G C C T G C T G G A G C T G A G T G C A T C C A A
mScn10a cds (GRI)   T C A T C G T C A C C G T G A G C C T G C T G G A G C T G A G C A C A T C C A A

                                2250                2260                2270                2280
Y09108-cds          A A A G G G C A G C C T A T C T G T G C T C C G T T C C T T A C G C T T G C T T
mScn10a cds (GRI)   G A A G G G C A G C T T G T C T G T G C T C C G C A C C T T C C G C T T G C T T

                                2290                2300                2310                2320
Y09108-cds          C G G G T C T T C A A G C T G G C C A A G T C C T G G C C C A C C C T G A A C A
mScn10a cds (GRI)   C G G G T C T T C A A G C T G G C C A A G T C C T G G C C C A C C C T G A A C A

                                2330                2340                2350                2360
Y09108-cds          T G C T C A T C A A G A T C A T C G G G A A C T C T G T G G G G G C C C T G G G
mScn10a cds (GRI)   T G C T C A T C A A G A T C A T C G G G A A C T C T G T G G G G G C C C T G G G

                                2370                2380                2390                2400
Y09108-cds          C A A C C T G A C C T T C A T C C T G G C C A T C A T C G T C T T C A T C T T T
mScn10a cds (GRI)   C A A C C T G A C C T T C A T C C T G G C C A T C A T C G T C T T T A T C T T C

                                2410                2420                2430                2440
Y09108-cds          G C C C T G G T G G G A A A G C A G C T C C T C T C A G A G A A C T A T G G G T
mScn10a cds (GRI)   G C C C T G G T G G G A A A G C A G C T C C T C T C A G A G A A C T A T G G G T

                                2450                2460                2470                2480
Y09108-cds          G C C G C A G G G A T G G C G T C T C C G T G T G G A A T G G T G A G A A G C T
mScn10a cds (GRI)   G C C G C A G G G A T G G C A T C T C C G T G T G G A A T G G T G A G A G G C T

                                2490                2500                2510                2520
Y09108-cds          G C G C T G G C A C A T G T G T G A C T T C T T C C A T T C C T T C C T C G T C
mScn10a cds (GRI)   G C G C T G G C A C A T G T G T G A C T T C T T C C A T T C C T T C C T C G T C
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                                       2570      2580      2590      2600
Y09108-cds            GGGTCTGCATGGAGGTCAGCCAGAACTACATCTGCCTCAC
mScn10a cds (GRI)     GGGTCTGCATGGAGGTCAGCCAGGACTACATCTGCCTCAC

                                       2610      2620      2630      2640
Y09108-cds            CCTCTTCTTGACAGTGATGGTGCTAGGCAACCTGGTGGTG
mScn10a cds (GRI)     CCTCTTCTTGACAGTGATGGTGCTAGGCAACCTGGTGGTG

                                       2650      2660      2670      2680
Y09108-cds            CTCAACCTTTTTATCGCTTTACTGCTGAATTCCTTTAGTG
mScn10a cds (GRI)     CTCAACCTATTCATCGCTTTACTGCTGAACTCCTTCAGTG

                                       2690      2700      2710      2720
Y09108-cds            CGGACAACCTCACGGCCCCAGAGGATGACGGGGAGGTGAA
mScn10a cds (GRI)     CGGACAACCTCACAGCCCCAGAGGATGACGGGGAGGTGAA

                                       2730      2740      2750      2760
Y09108-cds            CAACTTGCAGTTAGCACTGGCCAGGATTCAGGTACTTGGC
mScn10a cds (GRI)     CAACTTGCAGGTAGCACTGGCCCGGATTCAGGTATTTGGC

                                       2770      2780      2790      2800
Y09108-cds            CATCGGGCCAGTCGGGCCATTACCAGTTACATCAGAAGCC
mScn10a cds (GRI)     CATCGGGCCAGTCGGGCCATTACCAGTTACATCAGAAGCC

                                       2810      2820      2830      2840
Y09108-cds            ATTGCCGGTTCCGTTGGCCCAAGGTGGAGACCCAGCTGGG
mScn10a cds (GRI)     ACTGCCGGCTCCGCTGGCCCAAGGTGGAGACCCAGCTGGG

                                       2850      2860      2870      2880
Y09108-cds            GATGAAACCCCCACTCACCAGCTGCAAAGTTGAGAACCAC
mScn10a cds (GRI)     GATGAAACCCCCACTCACCAGCTGCAAAGCTGAGAACCAC

                                       2890      2900      2910      2920
Y09108-cds            ATTGCTACTGATGCTGTCAATGCTGCAGTGGGGAACCTGA
mScn10a cds (GRI)     ATTGCTACTGATGCTGNCAATGCTGCAGTGGGGAACCAGG

                                       2930      2940      2950      2960
Y09108-cds            CAAAGCCAGCTCTTAGTGGCCCCAAGGAGAATCACGGGGA
mScn10a cds (GRI)     CAAAGCCAGCTCTTGGTGGCCCCAAGGAGAACCACGGGGA

                                       2970      2980      2990      3000
Y09108-cds            CTTCATCACTGATCCTAACGTGTGGGTCTCTGTGCCCATT
mScn10a cds (GRI)     CTTCATCACTGATCCTAACGTGTGGGTCTCTGTGCCCATT

                                       3010      3020      3030      3040
Y09108-cds            GCTGAGGGGGAGTCCGACCTTGATGAGCTCGAGGAAGATG
mScn10a cds (GRI)     GCTGAGGGGGAGTCCGACCTTGATGAGCTCGAGGAAGATG
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                               3090      3100      3110      3120
Y09108-cds           CAAAGGGCAGCAGGAGCTGCTGCCACAAGTCCAAAAGTGT
mScn10a cds (GRI)    CAAAGGGCAGNNNGAGCTGCTGCAGCAAGTCCAAAAGTGT

                               3130      3140      3150      3160
Y09108-cds           GAAAATCACCAGGCAGCCAGAAGCCCAACCTCCGGGATGT
mScn10a cds (GRI)    GAAGATCACCAGGCAGCCCGAAGCCCACCCTCCGGGATGT

                               3170      3180      3190      3200
Y09108-cds           CCTCTGAAGACCTGGCTCCATACCTGGGGGAGAGATGGAA
mScn10a cds (GRI)    CCTCTGAGGACCTGGCTCCATACCTGGGGGAGAGATGGCA

                               3210      3220      3230      3240
Y09108-cds           AAGGAAGGATAACCCTCAGGTCCCTGCCGAGGGAGTGGAT
mScn10a cds (GRI)    GAGGGAGGAGAGCCCTCGGGTCCCTGCCGAGGGAGTGGAT

                               3250      3260      3270      3280
Y09108-cds           GACACAAGCTCCTCCGAGGGCAGCACGGTGGACTGCCCGG
mScn10a cds (GRI)    GACACAAGCTCCTCCGAGGGCAGCACGGTGGACTGCCCGG

                               3290      3300      3310      3320
Y09108-cds           ACCCAGAGGAGATCCTGAGGAAGATCCCTGAGCTGGCGGA
mScn10a cds (GRI)    ACCCAGAGGAGATCCTGAGGAAGATCCCTGAGCTGGCGGA

                               3330      3340      3350      3360
Y09108-cds           GGATCTGGACGAGCCCGATGACTGTTTCACAGAAGGCTGC
mScn10a cds (GRI)    GGAGCTGGACGAGCCCGATGACTGTTTCCCAGAAGGCTGC

                               3370      3380      3390      3400
Y09108-cds           ACTCGCCGCTGTCCCTGCTGCAAAGTGAACACCAGTAAGT
mScn10a cds (GRI)    ACTCGCCGCTGTCCCTGCTGCAAAGTGAACACCAGTAAGT

                               3410      3420      3430      3440
Y09108-cds           CTCCTTGGGCCACAGGCTGGCAGGTGCGCAAAACCTGTTA
mScn10a cds (GRI)    TTCCTTGGGCCACGGGCTGGCAGGTGCGCAAAACCTGTTA

                               3450      3460      3470      3480
Y09108-cds           CCGCATTGTGGAGCACAGCTGGTTTGAGAGTTTTATCATC
mScn10a cds (GRI)    CCGCATTGTGGAGCACAGCTGGTTTGAGAGTTTCATTATC

                               3490      3500      3510      3520
Y09108-cds           TTCATGATCCTGCTCAGCAGTGGAACGCTGGCCTTTGAGG
mScn10a cds (GRI)    TTCATGATCCTGCTCAGCAGTGGAGCGCTGGCCTTTGAGG

                               3530      3540      3550      3560
Y09108-cds           ATAACTACCTGGAAGAGAAACCCCGAGTGAAGTCTGTGCT
mScn10a cds (GRI)    ATAACTACCTGGAAGAAAAGCCCCGAGTGAAGTCTGTGCT
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                      3610      3620      3630      3640
Y09108-cds            GAGATGTTGCTCAAGTGGGTAGCCTATGGCTTCAAAAAAT
mScn10a cds (GRI)     GAGATGTTGCTCAAGTGGGTAGCTTATGGCTTCAAAAAAT

                      3650      3660      3670      3680
Y09108-cds            ATTTCACCAATGCCTGGTGCTGGCTGGACTTCCTCATCGT
mScn10a cds (GRI)     ATTTCACCAATGCCTGGTGCTGGCTGGACTTCCTCATCGT

                      3690      3700      3710      3720
Y09108-cds            GAACATCTCCCTCACAAGCCTCATAGCCAAGATCCTCGAG
mScn10a cds (GRI)     GAATATCTCCCTCACAAGCCTCATAGCCAAGATCCTCGAG

                      3730      3740      3750      3760
Y09108-cds            TATTCAGACGTGGCGTCCATCAAAGCCCTTCGGACTCTCC
mScn10a cds (GRI)     TATTCAGACGTGGCGTCCATCAAAGCCCTTCGGACTCTCC

                      3770      3780      3790      3800
Y09108-cds            GTGCCCTCCGGCCGCTGCGGGCTCTGTCTCGATTCGAAGG
mScn10a cds (GRI)     GTGCCCTCCGGCCGCTGCGGGCTCTGTCTCGATTCGAAGG

                      3810      3820      3830      3840
Y09108-cds            CATGAGGGTAGTGGTGGATGCCTTGGTGGGCGCCATCCCC
mScn10a cds (GRI)     CATGAGGGTAGTGGTGGATGCCTTGGTGGGCGCCATTCCC

                      3850      3860      3870      3880
Y09108-cds            TCCATCATGAACGTCCTCCTCGTCTGCCTCATCTTCTGGC
mScn10a cds (GRI)     TCCATCATGAACGTCCTCCTCGTCTGCCTCATCTTCTGGC

                      3890      3900      3910      3920
Y09108-cds            TCATCTTCAGCATCATGGGTGTGAACCTCTTCGCCGGGAA
mScn10a cds (GRI)     TCATCTTCAGCATCATGGGTGTGAACCTCTTCGCCGGGAA

                      3930      3940      3950      3960
Y09108-cds            ATTTTCGAGATGTGTCGACACCAGAAGCAACCCATTTTCC
mScn10a cds (GRI)     ATTTTCGAGATGTGTCGACACCAGAAGCAACCCATTTTCC

                      3970      3980      3990      4000
Y09108-cds            GTCGTGAATTCGACATTCGTGAATAACAAGTCTGACTGTC
mScn10a cds (GRI)     GTCGTGAATTCGACATTCGTGACTAACAAGTCTGACTGTT

                      4010      4020      4030      4040
Y09108-cds            ACAATCAAAACAATACCGGCCACTTCTTCTGGGTTAACGT
mScn10a cds (GRI)     ACAATCAAAACAATACTGGCCACTTCTTCTGGGTTAACGT

                      4050      4060      4070      4080
Y09108-cds            CAAAGTCAACTTCGACAACGTCGCTATGGGCTACCTCGCG
mScn10a cds (GRI)     CAAAGTCAACTTCGACAACGTTGCTATGGGCTACCTCGCG
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                          4130      4140      4150      4160
Y09108-cds          TGTATGCAGCTGTCGATTCTCGAGATATCAACAGTCAGCC
mScn10a cds (GRI)   TGTATGCAGCTGTCGATTCTCGAGATATCAACAGTCAGCC

                          4170      4180      4190      4200
Y09108-cds          CAATTGGGAGGAGAGCCTGTACATGTACCTATACTTCGTC
mScn10a cds (GRI)   CAATTGGGAGGAGAGCCTGTACATGTACCTATACTTCGTC

                          4210      4220      4230      4240
Y09108-cds          GTCTTCATCATTTTCGGTGGCTTCTTCACGCTGAATCTCT
mScn10a cds (GRI)   GTCTTCATCATTTTCGGTGGCTTCTTCACGCTGAATCTCT

                          4250      4260      4270      4280
Y09108-cds          TTGTCGGGGTCATCATCGACAACTTCAATCAACAGAAAAA
mScn10a cds (GRI)   TTGTCGGGGTCATCATTGACAACTTCAATCAACAGAAAAA

                          4290      4300      4310      4320
Y09108-cds          AAAGTTGGGGGGCCAGGACATCTTCATGACGGAAGAACAA
mScn10a cds (GRI)   AAAGCTAGGGGGCCAGGACATCTTCATGACAGAGGAGCAG

                          4330      4340      4350      4360
Y09108-cds          AAAAAATATTACAATGCCATGAAAAAACTGGGCTCCAAAA
mScn10a cds (GRI)   AAGAAGTACTACAATGCCATGAAGAAGCTGGGCTCCAAGA

                          4370      4380      4390      4400
Y09108-cds          AACCCCAAAAGCCCATCCCACGGCCTCTGAATAAGTACCA
mScn10a cds (GRI)   AACCCCAGAAGCCCATCCCACGGCCTTTGAATAAGTACCA

                          4410      4420      4430      4440
Y09108-cds          GGGCTTCGTGTTTGACATTGTGACCAGGCAAGCCTTTGAC
mScn10a cds (GRI)   GGGCTTCGTGTTTGACATTGTGACCAGGCAAGCATTTGAC

                          4450      4460      4470      4480
Y09108-cds          ATCATCATCATGGTTCTCATCTGCCTCAACATGATCACCA
mScn10a cds (GRI)   ATCATCATCATGGCTCTCATCTGCCTCAACATGATCACCA

                          4490      4500      4510      4520
Y09108-cds          TGATGGTGGAGACCGACAATCAGAGCGAGGAGAAGACGAA
mScn10a cds (GRI)   TGATGGTGGAGACCGACAATCAGAGCGAGGAGAAGACGAA

                          4530      4540      4550      4560
Y09108-cds          GGTTCTGGGCAGAATCAACCAGTTTTTTGTGGCGGTCTTC
mScn10a cds (GRI)   GGTCCTGGGCAGAATCAACCAGTTCTTCGTGGCCGTCTTC

                          4570      4580      4590      4600
Y09108-cds          ACGGGCGAGTGTGTGATGAAAGTGTTCGCCCTGCGACAGT
mScn10a cds (GRI)   ACGGGCGAGTGTGTGATGAAGATGTTCGCCCTTCGGCAGT
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                          4650      4660      4670      4680
Y09108-cds          GGTGATTCTGTCCATTGCGAGTCTGTTGTTTTCTGCAATC
mScn10a cds (GRI)   GGTGATTCTGTCCATTTCTAGTCTGTTGTTTTCTGCGATC

                          4690      4700      4710      4720
Y09108-cds          CTTAAGTCACTAGAAAGTTACTTCTCCCCCACGTTCTTCC
mScn10a cds (GRI)   CTTAGCTCACTAGAAAGTTACTTCTCCCCCACGCTCTTAC

                          4730      4740      4750      4760
Y09108-cds          GCGTCATCCGTCTGGCCAGGATCGGCCGCATCCTCAGGCT
mScn10a cds (GRI)   GCGTCATCCGTCTGGCCAGGATCGGCCGCATCCTCAGGCT

                          4770      4780      4790      4800
Y09108-cds          GATTCGAGCAGCCAAGGGGATTCGCACGCTGCTCTTCGCC
mScn10a cds (GRI)   GATTCGAGCAGCCAAGGGGATTCGCACGCTGCTCTTCGCC

                          4810      4820      4830      4840
Y09108-cds          CTCATGATGTCCCTGCCCGCCCTCTTCAACATCGCTCTCC
mScn10a cds (GRI)   CTCATGATGTCCCTGCCCGCCCTCTTCAACATCGGCCTCC

                          4850      4860      4870      4880
Y09108-cds          TCCTCTTCCTCGTCATGTTCATCTACTCCATCTTCGGCAT
mScn10a cds (GRI)   TCCTCTTCCTCGTCATGTTCATCTACTCCATCTTCGGCAT

                          4890      4900      4910      4920
Y09108-cds          GGCCAGCTTCGCTAATGTCATAGATGAGGCTGGCATCGAC
mScn10a cds (GRI)   GACCAGCTTCGCTAATGTCATAGATGAGGCTGGCATCGAC

                          4930      4940      4950      4960
Y09108-cds          GACATGTTCAACTTCAAGACCTTTGGCAACAGCATGCTGT
mScn10a cds (GRI)   GACATGTTCAACTTCAAGACCTTTGGCAACAGCATGCTGT

                          4970      4980      4990      5000
Y09108-cds          GCCTTTTCCAGATCACCACGTCGGCTGGCTGGGATGGCCT
mScn10a cds (GRI)   GCCTTTTCCAGATCACCACGTCGGCTGGCTGGGATGGCCT

                          5010      5020      5030      5040
Y09108-cds          CCTCAGCCCCATCCTCAACACAGGACCCCCCTACTGCGAC
mScn10a cds (GRI)   CCTCAGCCCCATCCTCAACACAGGACCCCCCTACTGCGAC

                          5050      5060      5070      5080
Y09108-cds          CCCAACCGGCCCAACAGCAATGGCTCCAAGGGGAATTGTG
mScn10a cds (GRI)   CCCAACCGGCCCAACAGCAATGGCTCCAAGGGGAATTGTG

                          5090      5100      5110      5120
Y09108-cds          GAAGCCCAGCGGTGGGCATCCTCTTCTTCACCACCTACAT
mScn10a cds (GRI)   GAAGCCCAGCGGTGGGCATCCTCTTCTTCACCACCTACAT
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                         5170      5180      5190      5200
Y09108-cds          GTGATTCTGGAGAACTTCAATGTGGCCACAGAAGAGAGCA
mScn10a cds (GRI)   GTGATTCTGGAGAACTTCAATGTGGCCACAGAAGAGAGCA

                         5210      5220      5230      5240
Y09108-cds          CGGAGCCCCTGAGCGAGGACGACTTTGACATGTTCTATGA
mScn10a cds (GRI)   CGGAGCCCCTGAGCGAGGACGACTTTGACATGTTCTATGA

                         5250      5260      5270      5280
Y09108-cds          GACCTGGGAGAAGTTTGACCCGGAGGCCACCCAGTTCATT
mScn10a cds (GRI)   GACCTGGGAGAAGTTTGACCCGGAGGCCACCCAGTTCATT

                         5290      5300      5310      5320
Y09108-cds          GCCTTTTCTGCCCTCTCAGACTTTGCAGACACACTCTCCG
mScn10a cds (GRI)   GCCTTTTCTGCCCTCTCAGACTTTGCAGACACACTCTCTG

                         5330      5340      5350      5360
Y09108-cds          GCCCTCTTAGAATCCCAAAACCTAATCAGAATATATTAAT
mScn10a cds (GRI)   GCCCTCTTAGAATCCCAAAACCTAATCAGAATATATTAAT

                         5370      5380      5390      5400
Y09108-cds          CCAGATGGACCTGCCGTTGGTCCCCGGAGATAAGATCCAC
mScn10a cds (GRI)   CCAGATGGACCTGCCGTTGGTCCCCGGAGATAAGATCCAC

                         5410      5420      5430      5440
Y09108-cds          TGTTTGGACATCCTCTTTGCCTTCACAAAGAATGTCTTGG
mScn10a cds (GRI)   TGTTTGGACATCCTCTTTGCCTTCACAAAGAATGTCTTGG

                         5450      5460      5470      5480
Y09108-cds          GAGAATCTGGGGAGTTGGATTCTCTGAAGACTAATATGGA
mScn10a cds (GRI)   GAGAATCTGGGGAGTTGGATTCTCTGAAGACTAATATGGA

                         5490      5500      5510      5520
Y09108-cds          AGAGAAGTTTATGGCAACTAATCTTTCCAAAGCATCCTAT
mScn10a cds (GRI)   AGAGAAGTTTATGGCAACTAATCTTTCCAAAGCATCCTAT

                         5530      5540      5550      5560
Y09108-cds          GAACCAATAGCAACCACCCTCCGGTGCAAGCAGGAAGACA
mScn10a cds (GRI)   GAACCAATAGCAACCACCCTCCGGTGCAAGCAGGAAGACA

                         5570      5580      5590      5600
Y09108-cds          TCTCAGCCACCATTATTCAAAAGGCCTATCGGAACTACAT
mScn10a cds (GRI)   TCTCAGCCACCATTATTCAAAAGGCCTATCGGAACTACAT

                         5610      5620      5630      5640
Y09108-cds          GTTGCAACGCTCCTTGATGCTCTCCAACACCCTGCATGTG
mScn10a cds (GRI)   GTTGCAACGCTCCTTGATGCTCTCCAACCCCCTGCATGTG
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                         5690          5700          5710          5720
Y09108-cds          GCTATGTTACATTCATGGCAAATGACAACGGTGGGCTCCC
mScn10a cds (GRI)   GCTATGTTACATTCATGGCAAATGACAACGGTGGGCTCCC

                         5730          5740          5750          5760
Y09108-cds          AGACAAATCAGAAACTGCTTCTGCTACGTCTTTCCCACCA
mScn10a cds (GRI)   AGACAAATCGGAAACTGCTTCTGCTACGTCTTTCCCACCA

                         5770          5780          5790          5800
Y09108-cds          TCCTATGAAAGCGTCACCAGGGGCCTGAGTGACAGGGCCA
mScn10a cds (GRI)   TCCTATGACAGCGTCACCAGGGGCCTGAGTGACAGGGCCA

                         5810          5820          5830          5840
Y09108-cds          ACATTAACACATCTAGCTCAATGCAAAATGAAGATGAAGT
mScn10a cds (GRI)   ACATTAGCACATCTAGCTCAATGCAAAATGAAGATGAAGT

                         5850          5860          5870          5880
Y09108-cds          CACTGCTAAGGAAGGGAATAGCCCTGGACCTCAGTGA
mScn10a cds (GRI)   CACTGCTAAGGAAGGGAAGAGCCCTGGACCTCAGTGA
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Appendix C

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                        10        20        30        40
Y09108        MEFPFGSVGTTNFRRFTPESLAEIEKQIAAHRAAKKGRTK
mScn10a(GRI)  MEFPFGSVGTTNFRRFTPGSLAEIEKQIAAHRAAKKGRPK

                        50        60        70        80
Y09108        QRGQKDKSEKPRPQLDLKACNQLPRFYGELPAELVGEPLE
mScn10a(GRI)  QRGQKDKSEKPRPQLDLKACNQLPRFYGELPAELVGEPLE

                        90       100       110       120
Y09108        DLDPFYSTHRTFILLNKSRTISRFSATWALWLFSPFNLIR
mScn10a(GRI)  DLDPFYSTHRTFIVLDKSRTISRFSATWALWLFSPFNLIR

                       130       140       150       160
Y09108        RTAIKVSVHSWFSIFITVTILVNCVCMTRTDLPEKLEYVF
mScn10a(GRI)  RTAIKVSVHSWFSIFITVTILVNCVCMTRTDLPEKLEYAF

                       170       180       190       200
Y09108        TVVYTFEALIKILARGFCLNEFTYLRDPWNWLDFSVITLA
mScn10a(GRI)  TVVYTFEALIKILARGFCLNEFTYLRDPWNWLDFSVITLA

                       210       220       230       240
Y09108        YVGAAVDLRGISGLRTFRVLRALKTVSVIPGLKVIVGALI
mScn10a(GRI)  YVGAAIDLRGISGLRTFRVLRALKTVSVIPGLKVIVGALI

                       250       260       270       280
Y09108        HSVRKLADVTILTVFCLSVFALVGLQLFKGNLKNKCIKNG
mScn10a(GRI)  HSVRKLADVTILTVFCLSVFALVGLQLFKGNLKNKCIKNG

                       290       300       310       320
Y09108        TDPHKADNLSSEMAEDIFIKPGTTDPLLCGNGSDAGHCPN
mScn10a(GRI)  TDPHKADNLSSEMAGDIFIKPGTTDPLLCGNGSDAGHCPN

                       330       340       350       360
Y09108        GYVCQKTPDNPDFNYTSFDSFAWAFLSLFRLMTQDSWERL
mScn10a(GRI)  DYVCRKTSDNPDFNYTSFDSFAWAFLSLFRLMTQDSWERL

                       370       380       390       400
Y09108        YQQTLRASGKMYMVFFVLVIFLGSFYLVNLILAVVTMAYE
mScn10a(GRI)  YQQTLRASGKMYMVFFVLVIFLGSFYLVNLILAVVTMAYE

                       410       420       430       440
Y09108        EQSQATIAEIEAKEKKFQEALEVLQKEQEVLAALGIDTTS
mScn10a(GRI)  EQSQATIAEIEAKEKKFKEALEVLQKEQEVLAALGIGTTS
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                         490                 500                 510                 520
Y09108          Y N Q R R M S F L G L S S G R R R A S H G S V F H F R A P S Q D V S F P D G I L
mScn10a(GRI)    Y N Q R R M S F L G L S S G R R R A S H S S V F H F R A P S Q D V S F P D G I L

                         530                 540                 550                 560
Y09108          D D G V F H G D Q E S R R N S I L L G R G A G Q A G P L P R S P L P Q S P N P G
mScn10a(GRI)    D D G V F H G D Q E S R R S S I L L G R G A G Q A G P L P R S P L P Q S P N P G

                         570                 580                 590                 600
Y09108          R K H G K E G Q L G M P T G E L A A G T P E G P A L D A A G Q K N F L S A G Y L
mScn10a(GRI)    P R R G E E G Q R G V P T G E L A T G A P E G P A L D A A G Q K N F L S A D Y L

                         610                 620                 630                 640
Y09108          N E P F R A Q R A M S V V S I M T S V I E E L E E S K L K C P P C L I S F A Q K
mScn10a(GRI)    N E P F R A Q R A M S V V S I M T S V I E E L E E S K L K C P P C L I S L A Q K

                         650                 660                 670                 680
Y09108          Y L I W E C C P K W R K F K M V L L E L V T D P F A E L T I T L C I V V N T V F
mScn10a(GRI)    Y L I W E C C P K W K K F K M V L F E L V T D P F A E L T I T L C I V V N T V F

                         690                 700                 710                 720
Y09108          M A M E H Y P M T D A F D A M L Q A G N I V F T V F F T M E M A F K I I A F D P
mScn10a(GRI)    M A M E H Y P M T D A F D A M L Q A G N I V F T V F F T M E M A F K I I A F D P

                         730                 740                 750                 760
Y09108          Y Y Y F Q K K W N I F D C V I V T V S L L E L S A S K K G S L S V L R S L R L L
mScn10a(GRI)    Y Y Y F Q K K W N I F D C V I V T V S L L E L S T S K K G S L S V L R T F R L L

                         770                 780                 790                 800
Y09108          R V F K L A K S W P T L N M L I K I I G N S V G A L G N L T F I L A I I V F I F
mScn10a(GRI)    R V F K L A K S W P T L N M L I K I I G N S V G A L G N L T F I L A I I V F I F

                         810                 820                 830                 840
Y09108          A L V G K Q L L S E N Y G C R R D G V S V W N G E K L R W H M C D F F H S F L V
mScn10a(GRI)    A L V G K Q L L S E N Y G C R R D G I S V W N G E R L R W H M C D F F H S F L V

                         850                 860                 870                 880
Y09108          V F R I L C G E W I E N M W V C M E V S Q N Y I C L T L F L T V M V L G N L V V
mScn10a(GRI)    V F R I L C G E W I E N M W V C M E V S Q D Y I C L T L F L T V M V L G N L V V

                         890                 900                 910                 920
Y09108          L N L F I A L L L N S F S A D N L T A P E D D G E V N N L Q L A L A R I Q V L G
mScn10a(GRI)    L N L F I A L L L N S F S A D N L T A P E D D G E V N N L Q V A L A R I Q V F G

                         930                 940                 950                 960
Y09108          H R A S R A I T S Y I R S H C R F R W P K V E T Q L G M K P P L T S C K V E N H
mScn10a(GRI)    H R A S R A I T S Y I R S H C R L R W P K V E T Q L G M K P P L T S C K A E N H
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                          1010                1020                1030                1040
Y09108          A E G E S D L D E L E E D V E Q A S Q S S W Q E E S P K G Q Q E L L P Q V Q K C
mScn10a(GRI)    A E G E S D L D E L E E D V E H A S Q S S W Q E E S P K G Q - E L L Q Q V Q K C

                          1050                1060                1070                1080
Y09108          E N H Q A A R S P T S G M S S E D L A P Y L G E R W K R K D N P Q V P A E G V D
mScn10a(GRI)    E D H Q A A R S P P S G M S S E D L A P Y L G E R W Q R E E S P R V P A E G V D

                          1090                1100                1110                1120
Y09108          D T S S S E G S T V D C P D P E E I L R K I P E L A E D L D E P D D C F T E G C
mScn10a(GRI)    D T S S S E G S T V D C P D P E E I L R K I P E L A E E L D E P D D C F P E G C

                          1130                1140                1150                1160
Y09108          T R R C P C C K V N T S K S P W A T G W Q V R K T C Y R I V E H S W F E S F I I
mScn10a(GRI)    T R R C P C C K V N T S K F P W A T G W Q V R K T C Y R I V E H S W F E S F I I

                          1170                1180                1190                1200
Y09108          F M I L L S S G T L A F E D N Y L E E K P R V K S V L E Y T D R V F T F I F V F
mScn10a(GRI)    F M I L L S S G A L A F E D N Y L E E K P R V K S V L E Y T D R V F T F I F V F

                          1210                1220                1230                1240
Y09108          E M L L K W V A Y G F K K Y F T N A W C W L D F L I V N I S L T S L I A K I L E
mScn10a(GRI)    E M L L K W V A Y G F K K Y F T N A W C W L D F L I V N I S L T S L I A K I L E

                          1250                1260                1270                1280
Y09108          Y S D V A S I K A L R T L R A L R P L R A L S R F E G M R V V V D A L V G A I P
mScn10a(GRI)    Y S D V A S I K A L R T L R A L R P L R A L S R F E G M R V V V D A L V G A I P

                          1290                1300                1310                1320
Y09108          S I M N V L L V C L I F W L I F S I M G V N L F A G K F S R C V D T R S N P F S
mScn10a(GRI)    S I M N V L L V C L I F W L I F S I M G V N L F A G K F S R C V D T R S N P F S

                          1330                1340                1350                1360
Y09108          V V N S T F V N N K S D C H N Q N N T G H F F W V N V K V N F D N V A M G Y L A
mScn10a(GRI)    V V N S T F V T N K S D C Y N Q N N T G H F F W V N V K V N F D N V A M G Y L A

                          1370                1380                1390                1400
Y09108          L L Q V A T F K G W M D I M Y A A V D S R D I N S Q P N W E E S L Y M Y L Y F V
mScn10a(GRI)    L L Q V A T F K G W M D I M Y A A V D S R D I N S Q P N W E E S L Y M Y L Y F V

                          1410                1420                1430                1440
Y09108          V F I I F G G F F T L N L F V G V I I D N F N Q Q K K K L G G Q D I F M T E E Q
mScn10a(GRI)    V F I I F G G F F T L N L F V G V I I D N F N Q Q K K K L G G Q D I F M T E E Q

                          1450                1460                1470                1480
Y09108          K K Y Y N A M K K L G S K K P Q K P I P R P L N K Y Q G F V F D I V T R Q A F D
mScn10a(GRI)    K K Y Y N A M K K L G S K K P Q K P I P R P L N K Y Q G F V F D I V T R Q A F D
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                      1530      1540      1550      1560
Y09108         T G E C V M K V F A L R Q Y Y F T N G W N V F D F I V V I L S I A S L L F S A I
mScn10a(GRI)   T G E C V M K M F A L R Q Y Y F T N G W N V F D F I V V I L S I S S L L F S A I

                      1570      1580      1590      1600
Y09108         L K S L E S Y F S P T F F R V I R L A R I G R I L R L I R A A K G I R T L L F A
mScn10a(GRI)   L S S L E S Y F S P T L L R V I R L A R I G R I L R L I R A A K G I R T L L F A

                      1610      1620      1630      1640
Y09108         L M M S L P A L F N I A L L L F L V M F I Y S I F G M A S F A N V I D E A G I D
mScn10a(GRI)   L M M S L P A L F N I G L L L F L V M F I Y S I F G M T S F A N V I D E A G I D

                      1650      1660      1670      1680
Y09108         D M F N F K T F G N S M L C L F Q I T T S A G W D G L L S P I L N T G P P Y C D
mScn10a(GRI)   D M F N F K T F G N S M L C L F Q I T T S A G W D G L L S P I L N T G P P Y C D

                      1690      1700      1710      1720
Y09108         P N R P N S N G S K G N C G S P A V G I L F F T T Y I I I S F L I V V N M Y I A
mScn10a(GRI)   P N R P N S N G S K G N C G S P A V G I L F F T T Y I I I S F L I V V N M Y I A

                      1730      1740      1750      1760
Y09108         V I L E N F N V A T E E S T E P L S E D D F D M F Y E T W E K F D P E A T Q F I
mScn10a(GRI)   V I L E N F N V A T E E S T E P L S E D D F D M F Y E T W E K F D P E A T Q F I

                      1770      1780      1790      1800
Y09108         A F S A L S D F A D T L S G P L R I P K P N Q N I L I Q M D L P L V P G D K I H
mScn10a(GRI)   A F S A L S D F A D T L S G P L R I P K P N Q N I L I Q M D L P L V P G D K I H

                      1810      1820      1830      1840
Y09108         C L D I L F A F T K N V L G E S G E L D S L K T N M E E K F M A T N L S K A S Y
mScn10a(GRI)   C L D I L F A F T K N V L G E S G E L D S L K T N M E E K F M A T N L S K A S Y

                      1850      1860      1870      1880
Y09108         E P I A T T L R C K Q E D I S A T I I Q K A Y R N Y M L Q R S L M L S N T L H V
mScn10a(GRI)   E P I A T T L R C K Q E D I S A T I I Q K A Y R N Y M L Q R S L M L S N P L H V

                      1890      1900      1910      1920
Y09108         P R A E E D G V S L P K G G Y V T F M A N D N G G L P D K S E T A S A T S F P P
mScn10a(GRI)   P R A E E D G V S L P R E G Y V T F M A N D N G G L P D K S E T A S A T S F P P

                      1930      1940      1950      1960
Y09108         S Y E S V T R G L S D R A N I N T S S S M Q N E D E V T A K E G N S P G P Q N
mScn10a(GRI)   S Y D S V T R G L S D R A N I S T S S S M Q N E D E V T A K E G K S P G P Q N
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Appendix D

Activation properties of Scn10a channels heterologously expressed in sympathetic neurons

Cell	1	2	3	4	5	6	7	8	9			
mV					Normalized Conductance					Mean	SEM	N
-39	-0.003	-0.005	-0.001	-0.001	-0.003	-0.001	-0.003	-0.004	-0.004	-0.003	0.000	9
-34	-0.004	-0.006	-0.001	-0.003	-0.003	-0.001	-0.004	-0.004	-0.004	-0.003	0.001	9
-29	-0.005	-0.006	-0.002	-0.001	-0.004	0.000	-0.005	-0.005	-0.005	-0.003	0.001	9
-24	-0.003	-0.008	-0.002	-0.002	-0.001	0.002	-0.005	-0.003	-0.004	-0.003	0.001	9
-19	0.000	-0.004	0.000	0.000	0.003	0.006	-0.003	-0.002	-0.003	0.000	0.001	9
-14	0.011	0.006	0.006	0.011	0.015	0.025	0.005	0.005	0.003	0.010	0.002	9
-9	0.049	0.035	0.021	0.026	0.047	0.086	0.023	0.016	0.014	0.035	0.008	9
-4	0.153	0.122	0.068	0.064	0.111	0.203	0.091	0.044	0.051	0.101	0.018	9
1	0.343	0.294	0.179	0.152	0.236	0.353	0.235	0.125	0.154	0.230	0.028	9
6	0.554	0.529	0.346	0.280	0.396	0.501	0.430	0.268	0.322	0.403	0.036	9
11	0.722	0.750	0.508	0.431	0.558	0.625	0.624	0.439	0.502	0.573	0.038	9
16	0.872	0.905	0.661	0.575	0.718	0.736	0.775	0.599	0.664	0.723	0.038	9
21	0.963	1.001	0.782	0.727	0.847	0.822	0.896	0.752	0.793	0.843	0.031	9
26	1.011	0.958	0.890	0.837	0.919	0.909	0.965	0.868	0.900	0.917	0.018	9
31	0.966	0.753	0.978	0.934	0.972	1.004	0.993	0.957	0.984	0.949	0.025	9

Boltzmann equation parameters as determined by nonlinear regression

Vh	5.07	5.42	11.21	13.49	9.33	6.90	8.12	13.16	11.43	9.35	1.06	9
k	5.43	4.75	6.73	7.26	6.72	7.81	5.74	6.53	6.30	6.36	0.31	9

Inactivation properties of Scn10a channels heterologously expressed in sympathetic neurons

Cell	1	2	3	4			
mV		Normalized conductance			Mean	SEM	N
-59	1.000	1.000	1.000	1.000	1.000	0.000	4
-53	1.011	0.998	1.001	0.985	0.999	0.005	4
-48	0.960	0.992	0.952	0.976	0.970	0.009	4
-44	0.956	0.963	0.883	0.933	0.934	0.018	4
-38	0.936	0.918	0.772	0.840	0.866	0.038	4
-34	0.907	0.841	0.611	0.717	0.769	0.066	4
-28	0.825	0.723	0.432	0.539	0.630	0.089	4
-23	0.658	0.560	0.278	0.348	0.461	0.089	4
-18	0.388	0.347	0.145	0.147	0.257	0.065	4
-14	0.127	0.137	0.046	0.046	0.089	0.025	4
-9	0.027	0.034	0.017	0.023	0.025	0.004	4
-4	0.004	0.008	0.004	0.009	0.006	0.001	4
1	0.000	0.000	0.000	0.000	0.000	0.000	4

Boltzmann equation parameters as determined by nonlinear regression

Vh	-20.68	-22.51	-30.14	-27.66	-25.25	2.20	4
K	-4.30	-5.56	-6.52	-5.89	-5.57	0.47	4

Appendix E

Activation properties of TTX-R sodium channels in mouse DRG neurons

Cell	1	2	3	4	5	6	7	8	9			
mV					Normalized Conductance					Mean	SEM	N
-58	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.0007	0.0001	9
-48	0.000	-0.002	0.000	0.000	0.001	0.000	0.000	-0.001	0.000	-0.0001	0.0003	9
-43	0.001	-0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.0003	0.0002	9
-38	0.001	-0.001	0.000	0.002	0.004	-0.001	0.000	0.002	-0.001	0.0007	0.0005	9
-34	0.003	0.002	0.002	0.004	0.007	0.001	0.001	0.005	0.000	0.0028	0.0007	9
-28	0.006	0.007	0.006	0.009	0.010	0.009	0.005	0.009	0.002	0.0069	0.0008	9
-24	0.013	0.022	0.016	0.020	0.017	0.029	0.015	0.018	0.007	0.0175	0.0021	9
-19	0.032	0.057	0.046	0.047	0.032	0.082	0.048	0.042	0.022	0.0454	0.0058	9
-14	0.086	0.161	0.127	0.110	0.060	0.223	0.162	0.105	0.062	0.1217	0.0177	9
-9	0.244	0.373	0.312	0.268	0.134	0.437	0.433	0.271	0.184	0.2950	0.0349	9
-4	0.511	0.625	0.558	0.493	0.300	0.663	0.716	0.531	0.405	0.5335	0.0429	9
1	0.716	0.796	0.746	0.688	0.519	0.839	0.888	0.757	0.633	0.7313	0.0369	9
6	0.852	0.903	0.861	0.824	0.718	0.947	0.978	0.914	0.804	0.8668	0.0265	9
11	0.935	0.969	0.933	0.921	0.859	0.999	1.000	0.988	0.923	0.9475	0.0153	9
16	0.982	1.000	0.988	0.983	0.957	1.000	0.963	1.000	0.986	0.9843	0.0053	9
21	1.000	0.985	1.000	1.000	1.000	0.903	0.852	0.924	1.000	0.9628	0.0185	9

Boltzmann equation parameters as determined by nonlinear regression

Vh	-3.61	-6.20	-4.72	-3.38	0.72	-7.56	-7.73	-4.48	-1.69	-4.29	0.91	9
k	4.94	4.97	5.25	5.53	5.40	5.00	3.99	4.55	4.99	4.96	0.15	9

Inactivation properties of TTX-R sodium channels in mouse DRG neurons

Cell	1	2	3	4	5	6	7	8	9			
mV					Normalized Conductance					Mean	SEM	N
-58	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.0000	0.0000	9
-53	0.972	0.990	0.988	1.009	1.002	0.961	0.989	0.991	0.988	0.9877	0.0047	9
-48	0.919	0.963	0.944	0.996	1.013	0.913	0.952	0.965	0.950	0.9572	0.0108	9
-43	0.810	0.878	0.869	0.943	0.974	0.805	0.870	0.892	0.859	0.8777	0.0183	9
-38	0.625	0.759	0.732	0.815	0.875	0.620	0.697	0.726	0.663	0.7236	0.0282	9
-33	0.369	0.567	0.513	0.583	0.680	0.420	0.421	0.452	0.390	0.4885	0.0347	9
-29	0.140	0.321	0.266	0.272	0.377	0.222	0.158	0.184	0.148	0.2321	0.0277	9
-23	0.046	0.125	0.087	0.079	0.145	0.125	0.049	0.084	0.058	0.0888	0.0119	9
-19	0.023	0.035	0.032	0.033	0.050	0.078	0.023	0.060	0.035	0.0410	0.0061	9
-14	0.013	0.011	0.015	0.015	0.023	0.042	0.013	0.035	0.020	0.0210	0.0036	9
-9	0.003	0.003	0.006	0.005	0.010	0.021	0.005	0.021	0.007	0.0091	0.0023	9
-4	0.001	0.002	0.001	0.000	0.000	0.005	0.002	0.009	0.003	0.0025	0.0010	9
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.0000	9

Boltzmann equation parameters as determined by nonlinear regression

Vh	-36.20	-32.35	-33.04	-32.68	-30.89	-35.43	-35.16	-34.53	-35.62	-33.99	0.60	9
k	-4.71	-4.78	-4.41	-3.66	-3.83	-5.54	-3.82	-3.97	-3.95	-4.30	0.21	9

	20 · 40 · 60
	ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
9 4/25whole ▶	ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
A 4/25whole ▶	ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
B 4/25whole ▶	ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

	80 · 100 · 120
	TYCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
9 4/25whole ▶	TTCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
A 4/25whole ▶	TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
B 4/25whole ▶	TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

	140 · 160 · 180
	TCCTTCTCCYTCTCCTTCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
9 4/25whole ▶	TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCCCCTTCTCCTTCTCCTTCTCCTTCTCCTTC
A 4/25whole ▶	TCCTTCTCCTTCTCCTTCT...
B 4/25whole ▶	TCCTTCTCCCTCTCCTTCTCCTTCT...................................
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

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                                  200                 220                 240
                      :CCCTTTTCTCTGCCTCCTCCTCCTTCTCC:CCTCCTTCTTCTCCTTCCTCTTCTTCCTT

9 4/25whole ▶         TCCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT
A 4/25whole ▶         .CCCTTTTCTCTGCCTCCTCCTCCTTCTCCACCTCCTTCTTCTCCTTCCTCTTCTTCCTT
B 4/25whole ▶         .CCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀

                                  260                 280                 300
                      CTCTTCTTCTKWYYKCRKYSBYCCTTTCGAATGACKATSKMGSMMRYGACAACCAACAAT

9 4/25whole ▶         CTCTTCTTCTTTCTTCATTGCTCCTTTCGAATGACTATGTAGCAAACGACAACCAACAAT
A 4/25whole ▶         CTCTTCTTCTTTCTTCATTGTTCCTTTCGAATGACTATGTAGCAAATGACAACCAACAAT
B 4/25whole ▶         CTCTTCTTCTTTCTTCATTGTTCCTTTCGAATGACTATGTAGCAAATGACAACCAACAAT
Intron1-in/A-NotI ▶             GATCGCGGCCGCCCTTTCGAATGACTATGTAGC
Intron1-in/B-NotI ▶                                        GATCGCGGCCGCGACAACCAACAAT
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀

                                  320                 340                 360
                      CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGWYCCCAGAATTCCAA

9 4/25whole ▶         CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGACCCCAGAATTCCAA
A 4/25whole ▶         CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGTTCCCAGAATTCCAA
B 4/25whole ▶         CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGTCCCCAGAATTCCAA
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶   CAGCAACC
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
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	380 400 420
	ATGTCACRCACTTGCAGAAACTACCTGCAACCGACAAARTCTCGCCCCTGCTAGAGCAAG
9 4/25whole ▶	ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAGTCTCGCCCCTGCTAGAGCAAG
A 4/25whole ▶	ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAATCTCGCCCCTGCTAGAGCAAG
B 4/25whole ▶	ATGTCACGCACTTGCAGAAACTACCTGCAACCGACAAAATCTCGCCCCTGCTAGAGCAAG
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

	440 460 480
	AGGCAAATCATAGTCAGCTGCTGYGGNTCAATCYKNAAGCAGCTCCATATCCCACACCCG
9 4/25whole ▶	AGGCAAATCATAGTCAGCTGCTGTGG.TCAATCCG.AAGCAGCTCCATATCCCACACCCG
A 4/25whole ▶	AGGCAAATCATAGTCAGCTGCTGCGGGTCAATCTTGAAGCAGCTCCATATCCCACACCCG
B 4/25whole ▶	AGGCAAATCATAGTCAGCTGCTGTGG.TCAATCTG.AAGCAGCTCCATATCCCACACCCG
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

	500 520 540
	GGATTAAAACAGACATACTCATAATATTNCTGKGATTYTCTTCTTAAAGAAANGCAAAAT
9 4/25whole ▶	GGATTAAAACAGACATACTCATAATATTTCTGTGATTTTCTTCTTAAAGAAA.GCAAAAT
A 4/25whole ▶	GGATTAAAACAGACATACTCATAATATTTCTGGGATTCTCTTCTTAAAGAAAAGCAAAAT
B 4/25whole ▶	GGATTAAAACAGACATACTCATAATATT.CTGTGATTCTCTTCTTAAAGAAA.GCAAAAT
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

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                                 560                 580                 600
                   TTNYACTGCAATGAGGGAAAGATGNTCARAATTTATAAAGCNTAGTTTGTGGGGGAATGG
9 4/25whole ▶                    TTTCACTGCAATGAGGGAAAGATG.TCAAAATTTATAAAGC.TAGTTTGTGGGGGAATGG
A 4/25whole ▶                    TT.CACTGCAATGAGGGAAAGATG.TCAGAATTTATAAAGC.TAGTTTGTGGGGGAATGG
B 4/25whole ▶                    TTTTACTGCAATGAGGGAAAGATGGTCAAAATTTATAAAGCCTAGTTTGTGGGGGAATGG
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀

                                 620                 640                 660
                   GATGGAGCTTCTTACAAAGCAAGGAGNAARNCAATGGCTTCAGGGATATGGARCAAARAC
9 4/25whole ▶                    GATGGAGCTTCTTACAAAGCAAGGAGGAAA.CAATGGCTTCAGGGATATGGAGCAAAGAC
A 4/25whole ▶                    GATGGAGCTTCTTACAAAGCAAGGAG.AAAGCAATGGCTTCAGGGATATGGAGCAAAGAC
B 4/25whole ▶                    GATGGAGCTTCTTACAAAGCAAGGAGGAAG.CAATGGCTTCAGGGATATGGAACAAAAAC
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀

                                 680                 700                 720
                   ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGNNNCGGG
9 4/25whole ▶                    ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGG.CGGG
A 4/25whole ▶                    ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGGGCGGG
B 4/25whole ▶                    ACACCTGTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGG...CGGG
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀
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                                       740                 760                 780
                          NTATTGARGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG

9  4/25whole ▶            .TATTGAAGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
A  4/25whole ▶            .TATTGAGGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
B  4/25whole ▶            GTATTGAGGAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGG
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀
Intron1-out/B-SwaI/I-CeuI ◀

                                       800                 820                 840
                          ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGNTNNYTNCCTYMSRNNNGNK

9  4/25whole ▶            ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
A  4/25whole ▶            ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
B  4/25whole ▶            ACAGGAGGGTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAG
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀                                                                   GAG
Intron1-out/B-SwaI ◀                      GGTCAGGCTGTCACCACTCTCTGGATTT
Intron1-out/A-SwaI/I-CeuI ◀                                                            GAG
Intron1-out/B-SwaI/I-CeuI ◀               GGTCAGGCTGTCACCACTCTCTGGTTCGCTACCTTAGGACCGTT

                                       860                 880                 900
                          NKNRWWCSWTYYCCAAGAAGWWYKMNANSWTRGRRYYSYYNTNKKKRYSMKTNGGAACT

9  4/25whole ▶            TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
A  4/25whole ▶            TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
B  4/25whole ▶            TGTAAATCCTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACT
Intron1-in/A-NotI ▶
Intron1-in/B-NotI ▶
Intron1-out/A-SwaI ◀      TGTAAATCCTTCCCCAAGAAGATTT
Intron1-out/B-SwaI ◀
Intron1-out/A-SwaI/I-CeuI ◀ TGTAAATCCTTCCCCAAGAAGTTCGCTACCTTAGGACCGTTATAGTTACGATTT
Intron1-out/B-SwaI/I-CeuI ◀ ATAGTTACGATTT
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	920 940 960
	ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
9 4/25whole ▶	ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
A 4/25whole ▶	ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
B 4/25whole ▶	ACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGC
Intron1-in/A-NotI ▶	
Intron1-in/B-NotI ▶	
Intron1-out/A-SwaI ◀	
Intron1-out/B-SwaI ◀	
Intron1-out/A-SwaI/I-CeuI ◀	
Intron1-out/B-SwaI/I-CeuI ◀	

Appendix G:

Primers for 5' Rapid Amplification of cDNA Ends:

GCCAGCGACTCTGGAGTGAACCGTC- Scn10a5'RACE1
GACCAGCTCTGCTGGGAGCTCGC- Scn10a5'RACE2
GAACCTGGGCAGCTGGTTACAGGCC- Scn10a5'RACE3

RACE products

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Scn10a RACE Clone B from ATG out

Sequence Range: 1 to 245 5'UTR (245+ cds)

                10        20        30        40        50        60
      CCGGACAAGTGTAAGTTTCGCAGAGCTGGGGTCTCCAGCTTACTTCTGCTAATGCTACCC
      ____________________5' UTR MOUSE SCN10A_____________________>

                70        80        90       100       110       120
      CAGGCCTTTAGACGGAGAACAGATGGCAGATGGAGTTTCTTCCTGCCATGCGCGAATGCT
      ____________________5' UTR MOUSE SCN10A_____________________>

               130       140       150       160       170       180
      GAGCGGATCTCATGATCCCCGAGCTCATGGCTTTCAGTAGAGGCAACCTGGGCTAAGAAG
      ____________________5' UTR MOUSE SCN10A_____________________>

               190       200       210       220       230       240
      AGATCTCCGACTTACGGAGCAGCAAAGAAGAGTGTAAATCCTTCCCCAAGAAGAATGAGA
      ____________________5' UTR MOUSE SCN10A_____________________>

    >Start_codon
       |
       |
      AGATG
 GAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGCAGAGATCGAGA
 AGCAGATCGNTGCCCACCGCGCCGCCAAGAAGGGCAGACCTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAA
 GCCCAGGCCTCAGTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTC
```

```
Scn10a RACE Clone I from ATG out

Sequence Range: 1 to 209 5'UTR (210+ cds)

                10        20        30        40        50        60
      ACGCGGGGACTTCTGCTAATGCTACCCCAGGCCTTTAGACGGAGAACAGATGGCAGATGG
      _______________________SCN10A 5'UTR_________________________>

                70        80        90       100       110       120
      AGTTTCTTCCTGCCATGCGCGAATGCTGAGCGGGTCTCATGATCCCCGAGCTCATGGCTT
      _______________________SCN10A 5'UTR_________________________>

                                                 >splice_5'_end_of_exon
                                                        |
               130       140       150       160       170 |     180
      TCAGTAGAGGCAACCTGGGCTAAGAAGAGATCTCCGACTTACGGAGCAGCAAAGAGTGTA
      _______________________SCN10A 5'UTR_________________________>

                             >Start_codon
                                   |
```

```
            190       200         |
AATCCTTCCCCAAGAAGAATGAGAAGATG
___SCN10A 5'UT____>
```

GAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCCAGAGTCGCTGGCAGAGATCGAGAAGCAGA
CCGCTGCCCACCGCGCCGCCAAGAAGGGCAGACCTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTCA
GTTGGACTTGAAGGCCTGTAACCAGCTGCCCAGGTTC

Appendix H:

LM-PCR primers: used for LM-PCR out (upstream) walking into the first intron 5' from the translational start site: (simply used RACE primers on genomic template instead of cDNA)

GCCAGCGACTCTGGAGTGAACCGTC- Scn10a5'RACE1
GACCAGCTCTGCTGGGAGCTCGC- Scn10a5'RACE2
GAACCTGGGCAGCTGGTTACAGGCC- Scn10a5'RACE3

Sequence of LM-PCR product generated by walking into the first intron 5' from the translational start site:

```
          10        20        30        40        50        60
ATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC

          70        80        90       100       110       120
TTCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTCTCCTTC

         130       140       150       160       170       180
TCCTTCTCCTTCTCCTTCTCCTTCTCCTTCCCCTTCTCCTTCTCCTTCTCCTTCTCCTTC

         190       200       210       220       230       240
TCCCTTTTCTCTGCCTCCTCCTCCTTCTCCTCCTCCTTCTTCTCCTTCCTCTTCTTCCTT

         250       260       270       280       290       300
CTCTTCTTCTTTCTTCATTGCTCCTTTCGAATGACTATGTAGCAAACGACAACCAACAAT

         310       320       330       340       350       360
CAGCAACCTACCCATAGGGGCTCTAGAATTTATGTCCTCTGAGAGACCCCAGAATTCCAA

         370       380       390       400       410       420
ATGTCACACACTTGCAGAAACTACCTGCAACCGACAAAGTCTCGCCCCTGCTAGAGCAAG

         430       440       450       460       470       480
AGGCAAATCATAGTCAGCTGCTGTGGTCAATCCGAAGCAGCTCCATATCCCACACCCGGG

         490       500       510       520       530       540
ATTAAAACAGACATACTCATAATATTTCTGTGATTTTCTTCTTAAAGAAAGCAAAATTTT

         550       560       570       580       590       600
CACTGCAATGAGGGAAAGATGTCAAAATTTATAAAGCTAGTTTGTGGGGGAATGGGATGG

         610       620       630       640       650       660
AGCTTCTTACAAAGCAAGGAGGAAACAATGGCTTCAGGGATATGGAGCAAAGACACACCT

         670       680       690       700       710       720
GTGCTACCTGGATTTGTAGATGGACTGCAGAGGATGGAGGGGGGGGGGCGGGTATTGAA

         730       740       750       760       770       780
GAGCTGGAGACAGAGATAGGGGGCTGCTCTGTCAATCAAGTAGGGGTGATGGACAGGAGG

         790       800       810       820       830       840
GTTGCAGTGGTCAGGCTGTCACCACTCTCTGGTTATTTTCCTCCCAGAAGAGTGTAAATC

         850       860       870       880       890       900
CTTCCCCAAGAAGAATGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGAACTACCAACTT

         910       920
CAGACGGTTCACTCCAGAGTCGCTGGC
```

Appendix I:

LM-PCR primers: used for LM-PCR out (upstream) from the RACE products

CAGGTTGCCTCTACTGAAAGCCATGAGC –LMPCRScn10a-1
GCTCAGCATTCGCGCATGGCAGG –LMPCRScn10a-2
CTCCATCTGCCATCTGTTCTCCGTC –LMPCRScn10a-3
CTCCATCTGCCATCTGTTCTCCGTC –LMPCRScn10a-3a
CTCCGTCTAAAGGCCTGGGGTAGC –LMPCRScn10a

Sequence of combined two rounds of LM-PCR 5' from ends of RACE products:

```
        10        20        30        40        50        60
ATTCCAGTTGCTGAGTGGAGAGAGCACTGTAGGGTCATGGAAGGACAGTGGGGAGGTCTG

        70        80        90       100       110       120
TTAGAGGTCCTTGAAATTATATAGTGACCTCGCCATGATGGTGGTCTCAGAGATCGAGAG

       130       140       150       160       170       180
ATGATGTAATCAGGAGGACTCTAGGAATTCAAGTTAGAGGCCCCAGAAAGAGGGCTGTGG

       190       200       210       220       230       240
ACGAGGGACGGCTCTTGGATTACCTCTAGATGCTGGGCTTGTGAGTCCAGGCAAGCAGAG

       250       260       270       280       290       300
TGTTCTTGGAGAGGCTTCTCTGGGGGAGGATCATTCTGAGCAGGGCACAGGCACAGAAAT

       310       320       330       340       350       360
CATTAGTCCATCTGTAAACATGTCTGAGATGTTAGTGGAGTGTCCATGAAGGGAAATTCA

       370       380       390       400       410       420
GGCTTCTACCACATTAGTGTATATTTAAATCTGACACCAGGAGAGAGATTTATGATGGAG

       430       440       450       460       470       480
CTGACAGACTCCGGTGCCATGTCAGGTAGGTGACTGAAGCCCTGGGGAAGGAGAGGCGTA

       490       500       510       520       530       540
GGATGGAATCTTAAAACGATTCTCCAACTACTTCCAGGTGGCAGAGGAGGAGGCAGCCCA

       550       560       570       580       590       600
GGCCAGAGAAGCTCCTCTGAAAACAGAAGTCAAGAGGGTGGAGTGTGGTGCAAGGACCAT

       610       620       630       640       650       660
GCAGCTAATCCTGCGGAGCCCCTAGGATGAGAGCGCCAGAGAGGAGACACATGACACAGG

       670       680       690       700       710       720
GAGACCAGTAGAAACCTGTTAAGATTCCGGGTGTCTCAGGACTGCCTCTGGATGCACACT

       730       740       750       760       770       780
TCTTCCTTCTTGGGAAGTTACTTTTCTGTCACTGTGATGAAATACCTTAACCAAGGTGAC

       790       800       810       820       830       840
TCAAAGAAGAGAGGGTTTATCTGGGCTCACGGGTCCAGAGGTAGAGGAACACATGGAGAT

       850       860       870       880       890       900
CGTGGTGGGGAACCAGTGTAGCAGGCAAGCATGGTGGCTGGGGCTGAGGCTGAGAGCTTA

       910       920       930       940       950       960
TATCTTTGTCTGTATACAGAAAGCAGAGAGAGCCAACTGGGAATGACTTGTGGCTTTTGG
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       970       980       990      1000      1010      1020
AACCTGAAACCTGTCTTCGGTGACATGCTCCCTCCAGCGAAGGCAATGCCTCCTCAAACT

      1030      1040      1050      1060      1070      1080
CCCCAAAGGGCACCACAAACTAGGAACCAAGCACTCAGATGCCCGAGACTATGAGCGACA

      1090      1100      1110      1120      1130      1140
TCTCCTTCAGATCACCACACTTGGGTACACCCATTCTCCTGTCTCATCCAGTTTGCTCTT

      1150      1160      1170      1180      1190      1200
CTGGAAGGGTGGTGAGAGGGATGACAGCTAGTGACAAGTTGGAGAGACTTTAGAATAATT

      1210      1220      1230      1240      1250      1260
GCCATCACACAAAGCCTACCCTATCAGTTAGTGGCTGGCACGCTATCCCAACAGCTTGAG

      1270      1280      1290      1300      1310      1320
TCTGAACTTGCCAGAAATGGCCTCCGTCTCACCTCTCCCAGGCTCCCCAGCACCCACAGG

      1330      1340      1350      1360      1370      1380
TGCCCTCCCCCAAGACCTGACATCATCGGAGCACTGAAGAGATGCCTCCTCTGCCCCTTT

      1390      1400      1410      1420      1430      1440
CTCCCCTGGTCTGATTGCTACCAGGCAGCTGATCCACATGCCCTGCTCCAAGTTTGACCC

      1450      1460      1470      1480      1490      1500
CAGTCAGCAGGCTTCTCTGAAGAAGAGGGTCTGTTAGCATGACACACAGCATTTCCCATG

      1510      1520      1530      1540      1550      1560
CAACAGAACCTTGGAACCTAGGACAGAACTCAAGATATCAACGTGACACACACATGTACA

      1570      1580      1590      1600      1610      1620
TGTACCCTTACACACCTGAACGTGCATATACACACGTACACTTGTACACACACTAAAATA

      1630      1640      1650      1660      1670      1680
ACTGTGGTGCCAGGAGTGATCAGGGTACTGAGGTGACAGTCATTCACAAGGCTTGCCTTT

      1690      1700      1710      1720      1730      1740
CTCCACGTGACACACTTTGACCTTACCACCTGTTTCCACCTTGCTCTGGCATTTTAAAAA

      1750      1760      1770      1780      1790      1800
CATGACATTTTTAGTAAATTCTTTGAATTTTTTTGAGACAGGGTCTTACACTACAGCTCA

      1810      1820      1830      1840      1850      1860
AGCTGATCTTGGGTTTGCAGCAATCCTCTTCCCTCGACCACCCCCCCAACTAGGATGTGA

      1870      1880      1890      1900      1910      1920
GCTGCCATGCCCAGTTTGACTCTTTCCCAGATGTTTGTTTTTATTCTGTATGTATGAGTG

      1930      1940      1950      1960      1970      1980
TTCTCACTGTATGTATGTCTGTATATGCACCATGTAGAGCCCAAAAGCAGTTGCTGAATG

      1990      2000      2010      2020      2030      2040
CTCCGGAGCTTGGAGCTGTGGGTGGCTGTGAGCTGCCACGTGGGGTGCCAAGAATAGAAC

      2050      2060      2070      2080      2090      2100
ACAGGTCCTCTGCAAGAGCATCGAATGCTCTTAACCACTGAGCTATCCCTCCAGGTATTA

      2110      2120      2130      2140      2150      2160
AAACATAAAATAAGCTTGACTTTTTACTCTGACATAATTTAAAATTCACAAAAAGTTTTA

      2170      2180      2190      2200      2210      2220
AAAAAACGTAACAGCTTCCGTATACTCCCAATCCCATTTCCCCAGTTAGGATATTCTTTA

      2230      2240      2250      2260      2270      2280
ACCATAGTACATTGTCAAATGAGAAAACTAACATTCATACAACACGACTGATTTTGGTGA
```

2290 2300 2310 2320 2330 2340
AAATCCTATTTGGAGTACACTACCTTTGACTGTGATTTCTTTTTGCACTATGGCCCAGGC

2350 2360 2370 2380 2390 2400
TAGCCTGCAACTATTTATCATTATTTATCCCAGGGTGACTTGAACTTAGGGCAATTCTTC

2410 2420 2430 2440 2450 2460
TACCTCAGCTCTGCCCCCACTCCCACCTCAACTCCAGTCCTGGGGTTACAGGAGCAAGCC

2470 2480 2490 2500 2510 2520
ATCAAGTTCTATAACATTTAATACACAAGGACACTGGTTAAACTCAGAAGGACCTAAATT

2530 2540 2550 2560 2570 2580
AGCATAAGACTATGGGGACCAGAGAAGTGAGAAGTGAGGACAGGGGAGGAGGGCAGGGGA

2590 2600 2610 2620 2630 2640
GGGAAGATGGGAGGAATGATGGGAAGAGAATGAGAGAAGGCAGGGAGGGAGAGGAGAAGG

2650 2660 2670 2680 2690 2700
CCAGTGAAGGGAGAATGGGAAGGGAGGGAGTTGAGAGAAGGCAGGATCGGGAGCCATAGA

2710 2720 2730 2740 2750 2760
ATGTCTGTAGGAAACCATCAAAGGCATTTAATTTAATAAAGCAACCAGGATTGTACATAA

2770 2780 2790 2800 2810 2820
TTCTACTGTGTACATACAAACACTCAAGTTTTGGGAGCAAGAATTTTAGCTTTCCTTCCC

2830 2840 2850 2860 2870 2880
CTGCCCCTTTATGATTCAGTCTCTGCTAGAAAAAGTGGAGCCTTGCAGGGTGTGGTGGTG

2890 2900 2910 2920 2930 2940
CACGCCTTTAATTCCAGCGTTTGGGAGGCAGAGGCAGGTGGATTTCTGTGAGTTCCCGGT

2950 2960 2970 2980 2990 3000
CAACCAAATCTCCATAGTATGATCCTTCGTGGAATACCGGCCAACCAAACAAGCAAACAA

3010 3020 3030 3040 3050 3060
ACAAACAAAAATCCCAAACAAACCCCACCCCCACCCAAATAGAGGGGATTATTGACTCAA

3070 3080 3090 3100 3110 3120
AGAAGCCAATAATTTTGAGTTGGTTTGGGACATTTGAGTAAATGAAGCTGTAATGGGCAA

3130 3140 3150 3160 3170 3180
GCATGGGCCCTCGACAGTTTCCTGCAGTATAGCATGGCTTCCTAAGGCTGCGTGGGTTGC

3190 3200 3210 3220 3230 3240
ACTGTTACGGAGGGCTCAGCTCAGACAGGGGGTTCCCTGTGCAACCTCCTTTCTTATGGT

3250 3260 3270 3280 3290 3300
CCCACAACCCCACAGATAGGGCACTTTCCCTACCCAGCTCCCTTCTCGGCTCTCACTGGG

3310 3320 3330 3340 3350 3360
GTCGGAGAACATTTTTGTTTCAGCATTTCATCTGAAGCCACGGTTTCACATCATCAAGTC

3370 3380 3390 3400 3410 3420
TGCAAAAAAACCGTTCACAAACCACACCAGAACTTCTCGGTAAAGAACTCCTAAGACCAAA

3430 3440 3450 3460 3470 3480
GAGGGAGACTGGGTAGATTGTTTTTAATTTGTTTCTTTTTGTCAAAGGGGGACAAAACAC

3490 3500 3510 3520 3530 3540
GCTTTGGTGAGTGCGAGTGTTTATTCTGGGACACAAACCCAGAGTCTGGAAGGGAGCATT

3550 3560 3570 3580 3590 3600

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CAACGGGTGCTGCTCTGCCACGCAGGGGCAGCGGTGGGACTCAGCCCATCCTGCTAAGGA

        3610      3620      3630      3640      3650      3660
CGGGCAGCCTGAGCCAGGCTTGGGAGTCTGTCATGGCTGCCAGACGAATCATTATCTAAT

        3670      3680      3690      3700      3710      3720
TGCAGCCTTTTCTCTTCCTTAGGTTTCAGCAGGTCCCGAGAGAGCATTTAAAATCGCATT

        3730      3740      3750      3760      3770      3780
TACTACTTTACCATCTAATCACACATAAGCCTCTCCCTATACCCTCCACCCTCCTTCCAT

        3790      3800      3810      3820      3830      3840
TCAGAGTGTACTTTCTGGAGCCCATCCAGCAAGCAGGGTGGAACTCATGACGGGAAATGG

        3850      3860      3870      3880      3890      3900
GAACGGCGCCCACGAAGGCGTGATTCCTTGTAGATCCTTGAGTGATGGACGGGTGAGGTT

        3910      3920      3930      3940      3950      3960
TCCGTCAGGCAAGCCCAGCCACCTTCGTGGAGGAGCCCCGGACAAGTGTAAGTTTCGCAG

        3970      3980      3990      4000      4010      4020
AGCTGGGGTCTCCAGCTTACTTCTGCTAATGCTACCCCAGGCCTTTAGACGGAGAACAGA

        4030
TGGCAGATGGAG
```

Appendix J:

Primers for genomic screening of mouse library:

CCTGTGTGTGCTGTAAAAAGGATC - EX1-3' OUT
TGAGAAGATGGAGTTCCCCTTTGG – EX1-5' IN

Amplified fragment for PCR screening of mouse genomic library:

```
        10        20        30        40        50        60
TGAGAAGATGGAGTTCCCCTTTGGGTCCGTGGGAACTACCAACTTCAGACGGTTCACTCC

        70        80        90       100       110       120
AGAGTCGCTGGCAGAGATCGAGAAGCAGATCGCTGCCCACCGCGCCGCCAAGAAGGGCAG

       130       140       150       160       170       180
AACTAAGCAAAGAGGACAGAAGGACAAGAGTGAGAAGCCCAGGCCTCAGTTGGACTTGAA

       190       200       210       220       230       240
GGCCTGTAACCAGCTGCCCAGGTTCTATGGCGAGCTCCCAGCAGAGCTGGTCGGGGAGCC

       250       260       270
CCTGGAGGACCTGGATCCTTTTTACAGCACACACAGG
```

Souslova VA, Fox M, Wood JN, and Akopian AN "Cloning and Characterization of a Mouse Sensory Neuron Tetrodotoxin-Resistant Voltage-Gated Sodium Channel Gene, Scn10a" ***Genomics*** **41**, 201-209 (1997).